

COMPARITIVE EVALUATION OF THREE NOVEL MOUTHWASHES ON SALIVARY BUFFERING CAPACITY IN SCHOOL CHILDREN: AN INVIVO STUDY

¹P. PRATHYUSHA, ² SWATHI.B, ³ BOLISETTY ATULITHA, ⁴ K. SREE LAKSHMI, ⁵ K. NAVEEN KRISHNA, ⁶ P.K. SARASWATHI BAI

Professor and HOD, Department of pediatric and preventive dentistry, CKS Theja insitute of dental sciences and research, Renigunta road, Tirupati, Andhra Pradesh, India

Email id – prats67@gmail.com

Professor, Department of pediatric and preventive dentistry, CKS Theja insitute of dental sciences and research, Renigunta road, Tirupati, Andhra Pradesh, India

Email id – swathipedo@gmail.com

, Post graduate student, Department of pediatric and preventive dentistry, CKS Theja insitute of dental sciences and research, Renigunta road, Tirupati, Andhra Pradesh, India

Email id – atulithachandrasekar@gmail.com

Senior lecturer, Department of pediatric and preventive dentistry, CKS Theja insitute of dental sciences and research, Renigunta road, Tirupati, Andhra Pradesh, India

Email – srilakshmikedari810@gmail.com

Reader, Department of pediatric and preventive dentistry, CKS Theja insitute of dental sciences and research, Renigunta road, Tirupati, Andhra Pradesh, India

Email id – naveenkrishnakola@gmail.com

, Senior lecturer, Department of pediatric and preventive dentistry, CKS Theja insitute of dental sciences and research, Renigunta road, Tirupati, Andhra Pradesh, India

Email – Saraswathip.mds@gmail.com

DOI: <https://doi.org/10.63001/tbs.2026.v21.i02.pp373-380>

KEYWORDS

Mouthwash,
saliva,
buffering capacity,
fluoride,
hyaluronic acid,
nanosilver

Received on: 18-03-2026

Accepted on: 06-04-2026

Published on: 16-04-2026

Abstract

Background : Saliva's buffering capacity is the key factor in maintaining oral homeostasis. Despite fluoride mouthwash being the gold standard, its harmful effects have led to the development of newer formulations containing nanosilver, hyaluronic acid as they have superior antibacterial, remineralizing effects. Hence, the present study is aimed to compare the effects of these mouthwashes on buffering capacity of saliva.

Methods : This study included 66 healthy children aged 7 to 12 years. Salivary samples were collected from the participants, baseline salivary buffering capacity was measured using pH meter. Participants were divided into 3 equal groups as Group 1 received fluoride mouthwash, Group 2 received hyaluronic mouth wash, Group 3 received nanosilver mouthwash. All the participants were allowed to rinse mouthwashes for about 45 secs. Salivary samples were obtained again after 30 min for measuring the post intervention buffering capacity, and the same procedure was repeated for the period of 15 days.

Results : After 15 days, On intra group comparison, both fluoride and hyaluronic acid mouthwash groups showed a statistically significant increase in salivary buffering capacity ($p < 0.001$), whereas nanosilver group showed a non-significant change ($p > 0.05$). Intergroup comparison revealed a highly significant difference among all groups ($p < 0.001$), with hyaluronic acid mouthwash demonstrating greatest improvement, followed by nanosilver and fluoride. Post hoc analysis confirmed significant differences between all pairwise comparisons.

Conclusion : Hyaluronic acid mouthwash demonstrated superior effectiveness in improving buffering capacity compared to fluoride and nanosilver mouthwashes. It serves as a promising alternative for enhancing oral health in children.

INTRODUCTION:

Fluoride mouthwashes have been used extensively used in controlling caries, considered gold standard and widely recommended due to their proven efficacy in reducing plaque by reducing cariogenic bacterial activity enhancing enamel remineralization.¹ Additionally it also stabilised the pH fluctuations in oral cavity. However, concerns regarding excessive fluoride ingestion and the risk of dental fluorosis in younger children,² have prompted the exploration of many newer, natural alternative therapeutic agents like hyaluronic acid and nanosilver mouthwash, that are both equally effective and safe.

Hyaluronic acid, a naturally occurring glycosaminoglycan, biopolymer, anti oxidant known for its anti-inflammatory, antimicrobial, and remineralising properties contributes to improved gingival health and potentially supports a balanced oral environment.^{3,4}

Nanosilver, owing to its unique physicochemical properties, exhibits strong antimicrobial activity, against a broad spectrum of oral microorganisms with remineralising efficacy and effectively reduce oral microbial load⁵, thereby decreasing acid production and indirectly aiding in the maintenance of salivary pH and buffering efficiency with the added advantage of sustained action.⁶

Given the significance of salivary buffering capacity in maintaining oral homeostasis and preventing caries, and evaluating impact of these mouthwashes is a paramount importance. However, limited evidence of these mouthwashes on influencing

the salivary buffering capacity, the study was undertaken.

Therefore, the aim of the study was to assess and compare the effectiveness of these mouthwashes in improving salivary buffering capacity.

METHODS :

STUDY DESIGN :

The study was designed as a randomised clinical trial. The study was conducted in boarding school. The protocol of this study was revised and accepted by Ethics and Research committee of CKS Theja insitute of Dental sciences and Research (ref no : CKS/CAD/192/2026). Using G power analysis , the total sample size was calculated to be 66. Subjects were explained about the study and consent was obtained from the respective guardian. The inclusion criteria were taken as Cooperative children aged 7 to 12 yrs with good expectorating reflexes and subjects exhibiting caries ICDAS score of 2 to 6 with Sillness and Loe plaque index score of 0 to 2. Those who were having systemic diseases, special health care needs, and on any systemic medication were excluded.

METHODOLOGY :

All the subjects were explained about the procedure and were restrained from eating and drinking for 1 hour prior to saliva collection. They were asked to sit in upright position, 3ml of unstimulated saliva was collected gradually in a sterile container. Baseline buffering capacity was recorded by standard titration method by Ericsson which is the following method. 0.5ml volume of saliva is

mixed with a fixed volume of 1.5mL of 5mmol/L hydrochloric acid (HCl).⁷ The mixture is stirred for a uniform mix and then incubated for 20 minutes at room temperature. The pH was measured using calibrated digital pH meter. Further, 66 subjects were randomly divided into 3 groups using lottery method i.e; 22 in each group as Group I was allocated with fluoride mouthwash(Kidodent®, 0.05% sodium fluoride, Indoco Remedies Ltd., India), Group II was allocated with hyaluronic acid mouthwash (Sali®, sodium hyaluronate) and Group III with nanosilver mouthwash(NanoLife®, silver nanoparticles 10 ppm, India).

All these mouthwashes were diluted with water at a ratio of 1:2 in accordance with the manufacturer's instructions. Subjects in Group I with fluoride mouthwash, Group II with hyaluronic acid mouthwash, Group III with Nanosilver mouthwash were asked to rinse the mouthwashes for about 45 seconds. Post-intervention salivary samples were again obtained after 30 minutes interval to re-measure the buffering capacity. The same procedure was repeated for the period of 15 days. At end of study period the data was subjected to statistical analysis.

STATISTICAL ANALYSIS :

The data collected throughout the study was subjected to statistical

analysis using SPSS software version 21.0 (IBM Corp., Armonk, NY). It was used to calculate mean and standard deviation for each group. Intra group comparisons were made using paired t test, whereas intergroup comparisons was done using One way Anova and Posthoc tukey tests. The level of significance p-Value was set at $p \leq 0.05$.

RESULTS:

Table 1 and figure 1 shows the intra-group comparison of salivary buffering capacity before and after the use of different mouthwashes showed variable outcomes. In the fluoride mouthwash group, the mean salivary buffering capacity increased from 4.0364 ± 0.55186 (pre) to 5.3664 ± 0.31178 (post), which was found to be statistically significant ($t = -6.181$, $p < 0.001$).

Similarly, in the hyaluronic acid mouthwash group, the mean value increased from 3.9864 ± 1.51710 (pre) to 5.7636 ± 1.23877 (post), showing a highly significant difference ($t = -6.669$, $p < 0.001$). In contrast, the nano silver mouthwash group demonstrated an increase from 4.2455 ± 1.24595 (pre) to 5.0673 ± 1.18662 (post); however, this change was not statistically significant ($t = -1.242$, $p = 0.243$).

Table1 : intragroup comparison of buffering capacity using Paired t test

Groups	Intervals	Mean	Std.dev	tvalue	pvalue
Fluoride Mouthwash	Pre op	4.0364	0.55186	-6.181	0.000*
	Post op	5.3664	0.31178		
Hyaluronic acid mouth wash	Pre op	3.9864	1.5171	-6.669	0.000*
	Post op	5.7636	1.23877		
Nano silver mouth wash	Pre op	4.2455	1.24595	-1.242	0.243(NS)
	Post op	5.0673	1.18662		

FIGURE 1 : Intra group comparison of salivary buffering capacity

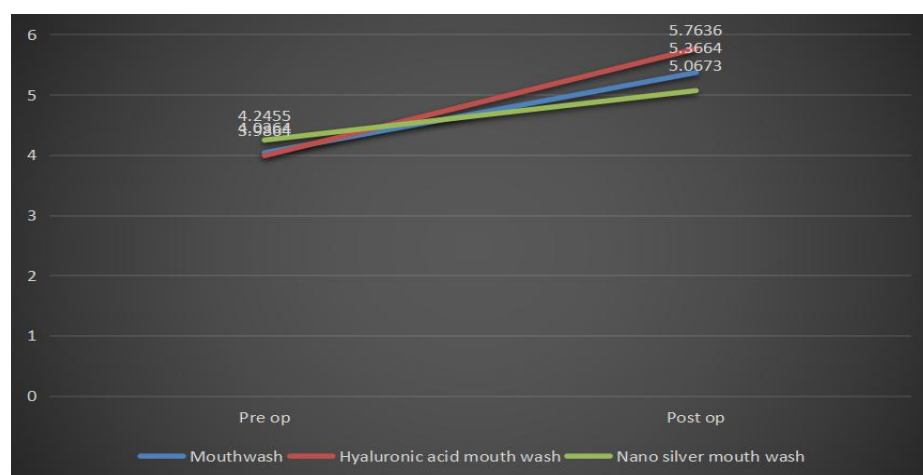


Table 2 and figure 2 shows The intergroup comparison of salivary buffering capacity among the three mouthwash groups. The hyaluronic acid mouthwash group demonstrated the highest mean value (1.7000 ± 0.00000), followed by the nano silver mouthwash group (0.7209 ± 0.03015) and the fluoride mouthwash group (0.3955 ± 0.01214). One-way ANOVA analysis revealed a highly statistically significant difference among the groups ($F = 14403.0$, $p < 0.001$). Overall, the hyaluronic acid mouthwash exhibited superior improvement in salivary buffering capacity compared to the other groups.

Table 2: Intergroup comparison of salivary buffering capacity based on mean difference at 15 days using Oneway Anova

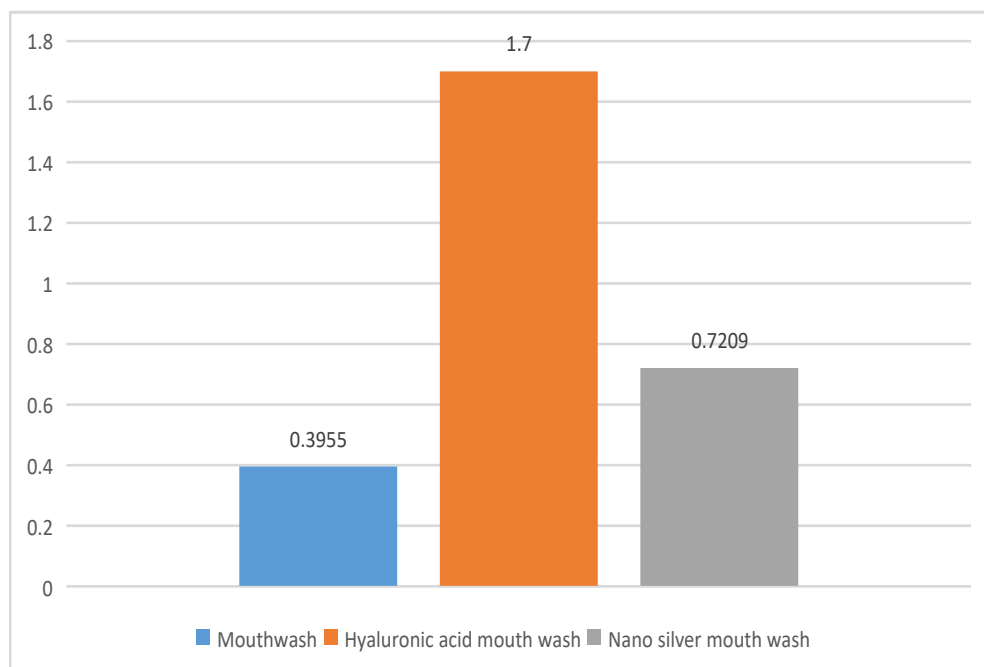
Groups	Mean	Std.dev	fvalue	pvalue
Fluoride Mouthwash	0.3955	0.01214	14403	0.000*
Hyaluronic acid mouth wash	1.7	0		
Nano silver mouth wash	0.7209	0.03015		

Table 3 shows the Post hoc pairwise comparison between the groups revealed that hyaluronic acid mouthwash group showed a significantly higher mean compared to the fluoride mouthwash group (*mean difference* = -1.30455, $p < 0.001$). Similarly, the nano silver mouthwash group demonstrated a significantly higher mean than the fluoride mouthwash group (*mean difference* = -0.32545, $p < 0.001$). Furthermore, the hyaluronic acid mouthwash group exhibited a significantly greater mean value compared to the nano silver mouthwash group (*mean difference* = 0.97909, $p < 0.001$).

Table 3 : multiple comparisons using posthoc tukey

GROUPS		MEAN DIFFERENCE	P VALUE
Fluoride mouth wash	Hyaluronic mouth wash	-1.30455*	0.000*
Fluoride mouth wash	Nanosilver mouth wash	-0.32545*	0.000*
Hyaluronic mouth wash	Nanosilver mouth wash	0.97909*	0.000*

FIGURE 2 : Intergroup comparison of salivary buffering capacity



DISCUSSION :

The present randomized clinical trial evaluated and compared the efficacy of fluoride, hyaluronic acid, and nanosilver mouthwashes on salivary buffering capacity among school children. Salivary buffering capacity is a key determinant in maintaining oral pH balance and preventing demineralization.⁸ As stated by Ericsson, salivary buffering systems play a decisive role in neutralizing plaque acids and protecting enamel integrity, highlighting its importance in caries prevention.⁹

In the current study, all three mouthwashes demonstrated an increase in salivary buffering capacity; however, the magnitude of improvement varied significantly. The fluoride mouthwash group showed a statistically significant increase, which aligns with previous findings by Featherstone, who reported that fluoride not only enhances remineralization but also inhibits bacterial acid production, thereby stabilizing oral pH.¹⁰

The hyaluronic acid mouthwash group exhibited the highest increase in buffering capacity among all groups, with highly significant results. This finding is in agreement with studies by Dahiya and Kamal described hyaluronic acid as a biocompatible agent with antimicrobial, anti-inflammatory, and wound healing properties that help maintain oral homeostasis.¹¹ Its hygroscopic nature and ability to retain water may enhance salivary function, thereby improving buffering efficiency. The superior performance of hyaluronic acid in this study suggests that it positively influences both microbial activity and salivary composition.

In contrast, the nanosilver mouthwash group showed an increase in buffering capacity that was not statistically significant in the intragroup comparison. Nanosilver is widely recognized for its antimicrobial efficacy. According to Rai et al., silver nanoparticles exhibit strong antibacterial activity against a wide range of microorganisms, including

oral pathogens.¹² Similarly, Morones et al., reported that nanosilver disrupts bacterial cell membranes and inhibits enzymatic activity, thereby reducing acidogenic potential.¹³ Despite these properties, the limited statistical significance observed in this study may be attributed to short duration, concentration differences, or variability in host response. However, intergroup analysis indicated that nanosilver performed better than fluoride, suggesting moderate effectiveness.

The intergroup comparison revealed that hyaluronic acid mouthwash was significantly more effective than both fluoride and nanosilver groups. These findings are consistent with emerging evidence suggesting that newer biologically active agents may offer broader therapeutic benefits. As noted by Gupta et al., non-fluoride agents with anti-inflammatory and antimicrobial properties are gaining importance as adjuncts in preventive dentistry, especially in pediatric populations.¹⁴

Post hoc analysis further confirmed statistically significant differences between all groups, indicating distinct mechanisms of action. From a clinical perspective, these findings are highly relevant. Although fluoride remains the gold standard in caries prevention, concerns about fluorosis in children necessitate safer alternatives. Marinho et al., stated that the benefits of fluoride must always be balanced against the risk of excessive ingestion in young children.¹⁵ In this context, hyaluronic acid emerges as a promising alternative due to its biocompatibility and absence of toxicity. Its superior efficacy in the present study supports its potential role as an adjunct or substitute for conventional mouthwashes in pediatric dentistry.

CONCLUSION :

Within the limitations of this study, all the three mouthwashes showed an improvement in salivary buffering capacity among school children. However, hyaluronic acid mouthwash demonstrated the most significant enhancement, followed by nanosilver and fluoride. These findings suggest that hyaluronic acid can be considered a promising and effective alternative, especially for short-term improvement in oral buffering capacity and preventive pediatric dental care. Future studies with larger sample sizes, longer follow-up, and additional biochemical assessments are recommended.

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