Comparative Evaluation of Chlorine Dioxide Based Mouth-rinse for the Management of Oral Halitosis in Children: A Clinical Study

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ABSTRACT

Aims & Background: Intra-oral halitosis, an unpleasant odor emanating from the mouth affecting a significant percentage of children globally. This study aims to compare the effectiveness of chlorine dioxide based mouth rinse against chlorhexidine and herbal mouth rinse in reducing oral halitosis among school-going children aged 8-12 years.

Materials and Methods: The study included 108 children aged 8–12 years with intra-oral halitosis of breath score below 3. The participants were randomly allocated into three groups *viz.* Group 1 (Herbal mouth-rinse), Group 2 (Chlorine-dioxide mouth-rinse), and Group 3 (Chlorhexidine mouth-rinse). Initially, the impact of three different mouth rinse groups on intra-oral halitosis was assessed for volatile sulphur compounds (VSCs) using gas chromatography before and after a single use. Secondly, the participants were assessed for their oral hygiene status based on oral hygiene indices followed by comparative assessment of bacterial colony counts using spread plate method at baseline and one week post treatment. The inhibitory effects of mouth rinses on tongue dorsum microbiota were measured through bacterial inhibition zones using well diffusion method.

Results: All the three mouth rinse groups showed significant ($p<0.001$) reductions in breath scores and VSCs levels with superior short-term efficacy of chlorhexidine. Chlorhexidine mouth rinse was most effective in reduction of VSCs score followed by chlorine dioxide and herbal oral rinse. Chlorhexidine also showed significant ($p<0.001$) reduction in oral hygiene indices and bacterial colony units compared to chlorine dioxide and herbal mouthrinse.

Conclusion: Chlorhexidine is the most effective intervention for oral halitosis management in children. However, chlorine dioxide and herbal mouth rinses offer viable alternatives.

Clinical significance: It is important to choose the right oral hygiene products and highlights the effectiveness of combining toothbrushing, tongue cleaning, and mouthwash like chlorine dioxide over brushing alone.

Keywords: Halitosis, Chlorine dioxide, Chlorhexidine Volatile sulphur compounds, Mouth rinses.

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INTRODUCTION

Halitosis, commonly known as bad breath or foetor oris, refers to an unpleasant odor originating from the oral cavity irrespective of its cause and is distinct from transient malodour caused by dietary factors, tobacco or medications.[1] It primarily results from microbial degradation of proteins producing volatile sulphur compounds (VSCs) mainly hydrogen sulphide (H_2S), methyl mercaptan (CH_3SH), and dimethyl sulphide [$(CH_3)_2S$]. Oral microorganisms including *Prevotella intermedia*, *Prevotella loescheii*, and *Fusobacterium periodonticum* have been associated with halitosis and periodontal disease, indicating a strong relationship between oral malodour and gingival inflammation.[2-4] The prevalence of halitosis among children is reported to be approximately 45% globally and 41% in India.[5] Dietary habits, plaque accumulation and inadequate oral hygiene practices contribute significantly to this condition among school going children. [6] Therefore, mouth rinses have gained importance as adjunctive

measures for plaque control and reduction of oral malodour, particularly in areas inaccessible to routine toothbrushing. [7]Chlorhexidine (CHX) remains the gold standard antimicrobial mouthwash due to its substantivity and proven efficacy; however, prolonged use may result in adverse effects including taste alteration, mucosal irritation, staining and calculus formation. [8] Chlorine dioxide-based oral rinses have demonstrated effectiveness in reducing oral malodour through oxidation of sulphur containing compounds and inhibition of VSC formation. Herbal mouth rinses containing medicinal plant extracts such as *Punica granatum* (pomegranate), *Azadirachta indica* (neem), *Ocimum sanctum* (tulsi) and *Camellia sinensis* (green tea) have also shown antimicrobial and antioxidant properties with fewer reported side effects. [9-11]

Considering the growing interest in both chemical and herbal approaches for halitosis management, the present study aimed to compare the inhibitory effect of chlorine dioxide, chlorhexidine and commercially available herbal mouth rinses in reducing oral halitosis among school going children.

MATERIALS AND METHODS

Study Design

The clinical study was conducted in the Department of Paediatric and Preventive Dentistry, and Department of Pharmacy, Ramaiah University of Applied Sciences, Bengaluru, Karnataka, following approval from the Institutional Ethics Committee ((EC-23/55-PG-FDS) and in accordance with the Declaration of Helsinki (1975, revised 2013). Written informed consent was obtained from parents prior to enrolment. Eligible participants were allocated into three groups: Group 1 received herbal mouth-rinse intervention (OraLife diluted in water in 1:1 ratio of 5mL), Group 2 received chlorine dioxide intervention (Freshclor: Sodium chlorite in stabilized form diluted with water in 1:2 ratio of 5mL), and and Group 3 received chlorhexidine intervention (Guard Oral Rinse: Chlorhexidine Gluconate Solution 0.2% w/v diluted with water in 1:1 ratio of 5mL).

Participants performed routine toothbrushing using the Modified Bass Technique and rinsed with the assigned mouthwash once daily for 3 minutes under supervision for one week. [12] Outcome assessments were carried out at baseline and after one week.

Participant selection

Inclusion criteria

Children aged 8 -12 years of either gender, presenting with a Kikar breath score ≥3, able to maintain oral hygiene independently, and exhibiting cooperative behaviour (Frankl behaviour rating scale score 3 or 4).

Exclusion criteria

Children with systemic illness, history of antibiotic use within the previous 3 months, known allergy to any component of the mouth rinses, or the presence of orthodontic appliances or prostheses**.**

Outcomes assessment

Stimulated saliva samples (5ml) were collected at baseline before lunch by asking participants to chew Xylitol gum and expectorate into labelled containers.[13] Following mouth rinse use, a second saliva sample was collected after 30 minutes for breath score assessment. [14] Samples labelled before (B) and after (A) rinsing were subjected to gas chromatography to determine volatile sulphur compound (VSC) concentrations. [15,16]

Tongue dorsum scrapings were collected and transported for microbiological analysis. The collected tongue scraping samples were cultured on enriched brucella blood agar to evaluate the bacterial inhibition zones. Following inoculation, four cylindrical wells, each measuring 7mm in diameter, were created in each culture plate. These wells were then filled with 60 µL of three different mouth-rinse formulations. The inoculated plates were placed in anaerobic jars and incubated for four days to allow bacterial growth under controlled conditions. After the incubation period, the diameter of the inhibition zones formed around each well was measured to assess the antibacterial efficacy of each mouth rinse against a standardized bacterial mixture. The differences in the mean inhibition zones among the two formulations were then analysed to compare their effectiveness in bacterial reduction.[17]

Clinical parameters including Oral Hygiene Index Simplified (OHIS), Gingival Index (GI), and Tongue Coating Index (TCI) were recorded to assess oral hygiene, gingival status, and tongue coating.

Statistical analysis

Data were entered in Microsoft Excel 2021 and analysed using IBM Statistical Software for Social Sciences (SPSS) version 22. Categorical variables were expressed as frequencies and percentages, while continuous variables were presented as mean ± standard deviation. One Way ANOVA with Tukey's *post-hoc* was used to compare the mean zone of Inhibition (ZOI) and oral indices (OHIS, GI, TCI–anterior, TCI–posterior). Non-normally distributed variables (Kikar Breath Score, VSC levels, and CFU counts) were analyzed using the Kruskal–Wallis test with Dunn's post-hoc test for intergroup comparisons and the Wilcoxon Signed-Rank test for within-group comparisons. Statistical significance was set at $p \leq 0.05$.

RESULTS

A total of 140 school-going children were initially screened for eligibility. Of these, 126 participants met

the inclusion criteria. Eighteen participants declined to participate and were excluded from the study. The remaining 108 eligible participants were randomly allocated into three experimental groups, with 36 participants in each group (n=36) (Figure 1). All three groups demonstrated a significant reduction in breath score from baseline to 30 minutes post-treatment. Group 1 showed a mean decrease from 3.72 to 1.58, Group 2 from 3.58 to 0.78, and Group 3 from 3.67 to 0.56. Intergroup comparison revealed a statistically significant ($p<0.001$) difference, with Group 3 exhibiting the most pronounced improvement in breath scores, indicating superior short-term efficacy (Table

Table 1: Comparison of mean Kikar breath score

Groups	Time	Mean ± SD	Mean Diff.	p-value
Group 1	Before Treatment	3.72 ± 0.45	2.14	<0.001
	After Treatment	1.58 ± 1.27		
Group 2	Before Treatment	3.58 ± 0.50	2.80	<0.001
	After Treatment	0.78 ± 0.76		
Group 3	Before Treatment	3.67 ± 0.48	3.11	<0.001
	After Treatment	0.56 ± 0.91		

Comparison of mean Kikar breath score between pre and post treatment in each group was done using Wilcoxon signed rank test

The mean VSC levels (mg/L) from baseline to 30 minutes post rinse showed significant ($p<0.001$) reduction within the groups with mean difference of 1.45mg/L, 3.4mg/L, and 3.3mg/L in Group 1, Group 2, and Group 3 respectively (Table 2).

Table 2: Comparison of mean volatile sulphur contents (VSCs)

Groups	Time	Mean ± SD	Mean Diff.	p-value
Group 1	Before Treatment	6.64 ± 1.79	1.45	<0.001
	After Treatment	5.19 ± 1.54		
Group 2	Before Treatment	7.29 ± 1.94	3.40	<0.001
	After Treatment	3.89 ± 1.81		
Group 3	Before Treatment	6.74 ± 1.57	3.33	<0.001
	After Treatment	3.41 ± 1.03		

Comparison of mean VSC levels between pre and post treatment in each group was done using Wilcoxon signed rank test

The OHI-S was 1.86 ± 0.76, the GI-L&GI-S score was 1.06 ± 0.42 for Group 2. Slightly poorer oral hygiene (OHI-S score: 1.25 ± 0.50) and more gingival inflammation (GI-L&S score: 0.96 ± 0.48) was observed in Group 3. Whereas, Group 1 has relatively good oral hygiene (mean OHI-S score: 1.06 ± 0.26) and mild gingival inflammation (GI-L&S score: 0.76 ± 0.42). The mean OHIS, GI-L & GI-S and TCI scores from baseline to 7 days post rinse were significantly ($p<0.001$) reduced. For all three groups the posterior third of the tongue showed the highest scores. Mean scores for all three groups were comparable, although Group 1 and Group 2 tended to have slightly higher scores (Table 3).

Table 3: Comparison of mean OHIS, GI-L & GI-S, TCI scores

Groups	Time	OHIS	GI	TCI-P	TCI-A	p-value
Group 1	Before Treatment	1.36 ± 0.76	1.16 ± 0.42	1.27 ± 0.61	1.23 ± 0.68	<0.001
	After Treatment	1.06 ± 0.26	0.76 ± 0.42	0.87 ± 0.61	1.11 ± 0.68	
Group 2	Before Treatment	1.86 ± 0.76	1.06 ± 0.42	1.17 ± 0.61	1.33 ± 0.68	<0.001
	After Treatment	1.21 ± 0.59	0.69 ± 0.47	0.58 ± 0.50	0.50 ± 0.51	

Group 3	Before Treatment	1.78 ± 0.59	1.57 ± 0.65	1.50 ± 0.61	1.61 ± 0.60	<0.001
	After Treatment	1.25 ± 0.50	0.96 ± 0.48	0.64 ± 0.49	0.64 ± 0.49	

Values were expressed as mean ± SD; Comparison of mean OHIS, GI-L & S, TCI between 2 groups Pre and Post Treatment in each Group using One-way ANOVA

OHIS, Oral Hygiene Index-Simplified; GI, Gingival index; TCI-P, Tongue coating index-Posterior; TCI-A, Tongue coating index-Anterior

Group 1's mean CFUs count decreased from 180.66 ± 21.07 to 128.00 ± 45.42, resulting in a mean difference of 52.65. Group 2's mean CFUs count decreased from 180.99 ± 49.58 to 87.49 ± 29.80, with a mean difference of 93.50. Group 3's mean CFUs count decreased from 175.31 ± 34.59 to 61.45 ± 28.99, with a mean difference of 113.85. The mean CFUs count before and after treatment for all three groups showed statistically significant ($p<0.001$) decreases in mean CFUs counts, with Group 3 experiencing the highest reduction (Table 4).

Table 4: Comparison of mean CFUs (log_{10})

Groups	Time	Mean ± SD	Mean Diff.	p-value
Group 1	Before Treatment	180.66 ± 21.07	52.65	<0.001
	After Treatment	128.00 ± 45.42		
Group 2	Before Treatment	180.99 ± 49.58	93.50	<0.001
	After Treatment	87.49 ± 29.80		
Group 3	Before Treatment	175.31 ± 34.59	113.85	<0.001
	After Treatment	61.45 ± 28.99		

Comparison of mean CFUs (log_{10}) between pre and post treatment in each group was done using Wilcoxon signed rank test

The Zone of Inhibition (ZOI) scores analyzed using Tukey's *post-hoc* test results showed significant differences in the among all the three groups (Table 5). Group 1 has the lowest ZOI compared to Group 2 (mean difference of -2.00) and Group 3 (mean of -3.64). Group 2 has a lower ZOI compared to Group 3 (mean difference of -1.64).

Table 5: Comparison of zone of inhibition (ZOI)

(I) Groups	(J) Groups	Mean Diff. (I-J)	95% CI for the Diff.		p-value
			Lower	Upper	
Group 1	Group 2	-2.00	-3.24	-0.76	<0.001
	Group 3	-3.64	-4.88	-2.40	<0.001
Group 2	Group 3	-1.64	-2.88	-0.40	0.004

Comparison of mean ZOI between groups using Tukey's *post-hoc* test

DISCUSSION

Mouth rinses play an adjunctive role in halitosis management through antimicrobial activity and reduction of volatile sulphur compounds (VSCs), with clinical effectiveness influences by their ability to reduce VSC producing microorganisms and modify the oral environment associated with malodour. [18,19] Due to limited evidence regarding chlorine dioxide use in children, the present study evaluated its efficacy in comparison with chlorhexidine and herbal mouth rinses. The present study demonstrated a significant reduction in halitosis following one week of mouth rinse use, as reflected by reductions in breath scores and VSC concentrations across all intervention groups. Since VSCs are considered the principal contributors to oral malodour, reduction in these compounds suggests improves control of halitosis. Gas chromatography was used in the present study to objectively quantify VSC levels, overcoming limitations associated with subjective organoleptic assessment. Similar findings were reported by Aung et al., who observed significant reduction in VSC levels following chlorine dioxide mouthwash use.[15-16] The reduction observed in the present study may be attributed to the oxidizing action of chlorine dioxide, which interferes with sulphur-containing amino acids and suppresses VSC formation. [20-22] Among the tested formulations, chlorine dioxide demonstrated efficacy comparable to chlorhexidine in reducing halitosis-related parameters including CH_3SH and $(CH_3)_2S$ concentrations. Although chlorhexidine remains the gold standard antimicrobial mouthwash, its clinical use is frequently limited by adverse effects such as

staining and altered taste.[8] The comparable outcomes observed in the present study suggest that chlorine dioxide may provide an effective alternative for short-term halitosis management. Similar findings have been reported in previous studies evaluating alternative and herbal mouth rinses for reducing oral malodour and improving oral health parameters.[23–25] In addition to reducing VSC levels, the present study demonstrated significant improvement in oral hygiene parameters including OHIS, GI, TCI-anterior, and TCI-posterior following intervention. Improvement in these indices suggests reduced plaque accumulation, lower gingival inflammation, and decreased tongue coating, which are recognized contributors to halitosis. Comparable reductions in oral hygiene indices following mouth-rinse use have been reported previously, supporting the relationship between improved oral hygiene and reduced oral malodour.[10,26] Microbiological findings further supported the clinical outcomes of the present study. Significant reductions in CFU counts and measurable antimicrobial activity through zone of inhibition (ZOI) analysis were observed after intervention. These findings indicate that reduction in bacterial load may have contributed to decreased production of malodorous metabolites. Similar antimicrobial effects have been demonstrated in studies evaluating chlorhexidine and other oral rinse formulations against oral microorganisms.[27–31] Although favourable short-term outcomes were observed, the present study was limited by the short duration of intervention and lack of long-term follow-up. Further studies involving larger paediatric populations are recommended to evaluate sustained effectiveness and clinical applicability.

Overall, the present findings indicate that chlorine dioxide mouth rinse demonstrated effective reduction of halitosis, improvement in oral hygiene indices, and antibacterial activity comparable to chlorhexidine, supporting its use as a promising adjunctive approach for halitosis management in children.

CONCLUSION

The present study demonstrated that chlorhexidine effectively reduced volatile sulphur compounds (VSCs), plaque accumulation and gingival inflammation, while chloride dioxide exhibited comparable antimicrobial efficacy with favourable short term outcomes in halitosis management. Herbal mouth rinses also showed beneficial effects in reducing oral malodour parameters. Therefore, chlorine dioxide and herbal mouth rinses may be considered potential alternatives to chlorhexidine for short term management off halitosis in children. Further long term studies are recommended to establish their sustained clinical effectiveness and safety.

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