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Antibacterial Efficacy of Benzalkonium Chloride Gel with Tongue Brush on S. Mutans and L. Acidophilus and Tongue Coating – A Randomized Controlled Trial

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Abstract:-

- ***Introduction:***

The tongue microbiota, like other parts of the oral cavity, plays a significant role in the development of dental caries. *S. mutans* and *L. acidophilus* species, are associated with the initiation and progression of dental caries.

- ***Aim:***

To assess the efficacy of benzalkonium Chloride gel along with tongue cleaning aids in the reduction of tongue coating and bacterial count on dorsal surface of the tongue.

- ***Materials and Methods:***

A double blinded randomized control trial was conducted among 17-25 years old college students. A total of 60 participants were randomly divided into 4 groups according to their use of tongue cleaning aids as follows: Group A, Tongue Brush Alone; Group B, Tongue Brush with Chlorhexidine Mouthwash (Clohex ADS); Group C, Tongue Brush with Chlorhexidine Gel (Hexigel); and Group D, Tongue Brush with Benzalkonium Chloride Gel. The Winkel tongue coating index (WTCI) was used to measure the tongue coating at baseline and at 14th day and the participant's tongue coatings on dorsal surface were sampled using swab method, and the number of *S. mutans* and *L. acidophilus* colonies were counted before and after intervention.

- ***Results:***

Both the Hexigel group and Benzalkonium chloride gel group reduced both the bacterial load and tongue coating at 14th Day compared to the baseline data.

- ***Conclusion:***

This study suggests that usage of Tongue brush along with Benzalkonium Chloride gel decreased the tongue coating and the number of bacteria on the dorsal tongue surface.

Keywords:- *Benzalkonium Chloride Gel, Tongue Coating, Tongue Brush, S. Mutans and L. Acidophilus.*

I. INTRODUCTION

Oral health is the state of the mouth, teeth and orofacial structures that enables individuals to perform essential functions such as eating, breathing and speaking, and encompasses psychosocial dimensions such as self-confidence, well-being and the ability to socialize and work without pain, discomfort and embarrassment. Dental health varies from childhood to old age, is essential to overall health, and helps people reach their full potential and engage in society.[1] However, the vital aspect of oral health is often overlooked, due to dental caries, making it as a significant public health concern.

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Dental caries, a prevalent childhood disease, results from the interplay between acidogenic bacteria and dietary carbohydrates.[2] It arises when the oral microbiota changes from a state of symbiotic equilibrium to dysbiosis. Host susceptibility in teeth and saliva further influences caries development, affecting both crowns and roots. Risk factors encompass biological, environmental, and behavioral aspects, including high cariogenic bacteria levels, low salivary flow, inadequate fluoride exposure, poor oral hygiene, inappropriate infant feeding practices, and socioeconomic status. According to the results of the most recent National Oral Health Survey, which was done in 2002–2003, the DMFT index score for Indian children was about 2, and the prevalence of caries was rising with age, rising from 51.9% to 63.1% in the age range of 5–15 years.[3] The oral cavity has been identified with more than 700 species of bacteria. A typical person may harbor between 100 and 200 different types of bacteria; this indicates a high degree of individual variation. The tongue, functioning as an initial interface within the digestive tract, harbors a diverse bacterial consortium. This unique ecological niche promotes bacterial colonization, potentially contributing to various health detriments. Normally, the esophagus carries the unattached microorganisms that are swallowed with saliva into the stomach, where they are inactivated by the stomach's acid and proteolytic enzymes.[4] Although aspirated saliva contains microorganisms colonizing in various sites, their bacterial composition indicates that the dominant source is the microbiota formed on the tongue. With its broad surface area and papillary structures, the tongue's dorsum may hold a variety of microorganisms, including both aerobes and anaerobes. The discharge of resident bacteria into the saliva is facilitated by the loose community structure and the desquamation of epithelial cells. These characteristics suggest that the tongue covering and bacteria need to be carefully considered.[5] Tongue coating refers to white, yellowish-brown, or black moss-like deposits on the tongue dorsum, which are caused by increased keratinization of cells on the tongue surface, elongation of lingual papillae, remnants of exfoliated epithelium, and food residue. It is affected by the functional state and amount of salivary gland secretions, resident bacteria in the oral cavity, and general systemic conditions. The quantity and quality of tongue coating may be affected by the presence of dry mouth, decreased immunity, oral respiration, poor oral hygiene, smoking, aging, stress, systemic diseases, and/or side effects of drugs.[6]

Due to its huge surface area, the dorsum of the tongue occupies a unique ecological niche. This niche is filled with food remnants, saliva, and degenerated epithelial cells, all of which can aid in the acquisition and proliferation of bacteria. The thickness of the tongue coating and oral malodor may be effectively decreased by using mechanical methods to clean the dorsum of the tongue, such as tongue scraping or brushing. A recent observational study assessed the preferences and efficacy of multiple commercially available tongue cleansers among the participants. The participants discovered that two scrapers were the most pleasant and efficient tongue cleansing goods when compared to other brands.[7]

Many commercially available mouthwash products contain Chlorhexidine, amine fluoride/stannous fluoride, and zinc lactate as antibacterial counteractive. Clinical trials evaluating their antibacterial effect have shown that this mouthwash was effective compared to a negative control.[8]

In addition, there are TUNG tongue gel and MERIDOL tongue gel available that contain zinc lactate as the major composition of commercially available mouthwash. One such antibacterial agent is Benzethonium Chloride. Benzalkonium chloride (purest form of Benzethonium Chloride), also known by several other names including alkyl-dimethyl-benzyl-ammonium chloride and Zephiran, is a quaternary ammonium compound that is widely used in a variety of products has anti-bacterial properties and is available in Hand sanitizers, Disinfectant wipes, Eye drops, Nasal sprays, Cosmetics, Soaps, and shampoos.[9] The clinical efficacy of using a tongue brush with benzalkonium chloride has not yet been evaluated. Therefore, the present clinical study was conducted to assess the antibacterial efficacy of benzalkonium Chloride gel along with tongue brush against tongue coating and bacterial count on dorsal surface of tongue.

II. MATERIALS AND METHODS

This double blinded randomized controlled trial, was designed to assess and compare the efficacy of Benzalkonium Chloride Gel, Clohex ADS and Hexigel on the dorsal surface of tongue among young adults aged 17 – 25 years of Madurai city. The nature and purpose of the study was explained to the Best Dental Science College Institutional Ethics Committee and ethical clearance was obtained to conduct the study. (BDSC-IEC/2024/MAY/P-20). Clinical Trial Registry – India Registration has been done before starting the study. (CTRI/2024/09/073813). Consolidated Standards for reporting trials (CONSORT guidelines) were followed. (Figure 1)

The method and purpose of the study were thoroughly explained to the study subjects. Written informed consent was procured from the subjects before the commencement of the study. Participation in this study was purely on voluntary basis and they were allowed to opt out from the study at any time they wish to do so. Participants who are willing to participate, without any systemic diseases, and Subjects having WTC index 1 at baseline are included in the study. Study participants who are smokers, alcoholics and having systemic diseases, participants on antibiotics one month prior to treatment, Patients having Bulimia, Subjects using any other oral hygiene aids, Subjects with Developmental Anomalies of tongue, Subjects allergic to any products used, Patients who are having piercing in the tongue, Women who are Pregnant are excluded from participating in the study. Based on the data obtained from the Pilot Study, the effect size was estimated as 0.57, and a total sample size of 60 is obtained, using G power software.

Multistage random sampling method was employed. Madurai is divided into 5 Zones, and One Zone (Zone IV) is picked randomly. There are 2 Catering colleges in Zone IV and 30 participants were selected from each college. The eligible study participants were randomly allocated using chit method.

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The participants were asked to blindly pick any of the four closed chits marked A, B, C and D and allotted into four groups.

- Group A, Tongue Brush Alone,
- Group B, Tongue Brush with Chlorhexidine Mouthwash (Clohex ADS),
- Group C, Tongue Brush with Benzalkonium Chloride Gel and
- Group D, Tongue Brush with Chlorhexidine Gel (Hexigel).

Clohex ADS mouthwash is packed in 20 ml spray bottles. The participants belonging to Group B are requested to spray 20 times over the bristles of tongue brush, so that the mouthwash moistens the tongue brush. The Gels were packed in an opaque container and were distributed in similar looking tubes. The palatability of the Benzalkonium Chloride gel was checked priorly by the investigator in case of uneasiness to the study subjects. In Group C and Group D, a Pea sized amount of corresponding gels is placed and requested to brush their tongue. Reinforcement of oral hygiene instructions to the study participants is done by phone calls and messages daily during the entire study period.

At the beginning of the study, the participants were given a new set of dental kit, containing a new set of toothbrushes (Colgate Flexible) and toothpaste (Colgate Total) and Tongue brush (Bamboo India), along with their respective products. After 15 minutes of brushing, the participants are requested to brush their tongue by X technique, which is demonstrated to patients after enrolling into the study.[11] Reinforcement of oral hygiene instructions to the study participants is done by phone calls and messages on daily basis during the entire study period. Baseline and final data, at 14th Day was collected by Winkel Tongue Coating Index scores (Winkel et al, 2003) and Bacterial Count by using the samples obtained from tongue swabs.[3] A sterile cotton swab was placed on the connected constant-pressure sample collecting device, positioned parallel to the back of the tongue, and rubbed back and forth in a 2-cm region located in the middle of the tongue dorsum in order to capture a sample from the tongue surface. The tongue coating samples were transported to the microbiological lab and incubated accordingly in Blood agar and MRS agar and the growth of the bacteria was identified and confirmed for *S. mutans* and *L. acidophilus.*

➢ *Preparation of Benzalkonium Chloride Gel*

Table 1 Ingredients Required for Preparation

Ingredients	**Values**
Carbopol 940	0.8 g
Benzalkonium Chloride	0.05%
Propylene Glycol 400	6ml
Pepper Mint Essential Oil	0.3ml
Xylitol	q.s.
Triethanolamine	q.s.
Distilled Water	q.s.

- Gel formulation solution was prepared by dissolving 0.8g Carbopol 940 in Distilled Water. Carbopol 940 which was soaked in water for 24hrs. Then it was stirred at 100rpm using a mechanical stirrer.
- Triethanolamine (Neutralizing agent) was added slowly to the beaker till it attained the gel structure. Measured quantity of 5g of 0.05% Benzalkonium Chloride was added followed by 6ml of propylene glycol 400 was added to the prepared gel and stirred continuously to obtain a proper gel form.
- To this required amount of Xylitol and Pepper Mint Essential Oil is added.[12]

➢ *Statistical Analysis*

The data from 60 study participants were analyzed with the help of computer using Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, version 20).

- *For Microbiological Analysis*

✓ Intergroup Comparison – The One way ANOVA test
✓ Intragroup Comparison – The Paired t-test

- *For Tongue Coating*

✓ Intergroup Comparison – The Kruskal Wallis H test
✓ Intragroup Comparison – The Wilcoxon Signed Rank test

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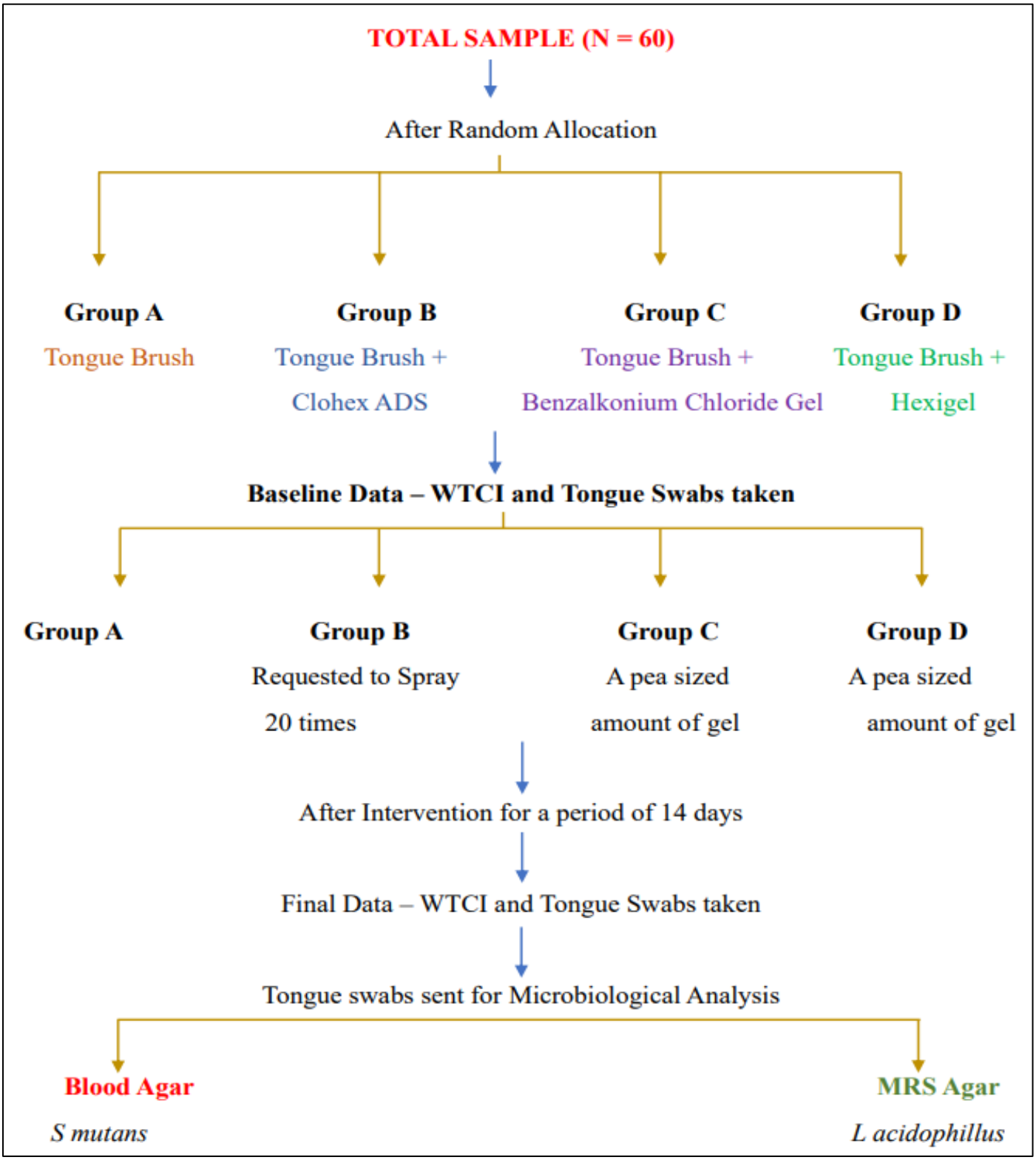

Fig 1 Consolidated Standards of Reporting Trials

III. RESULTS

Out of 60 total study participants, all the participants successfully completed the trial with full participation and none of the participants showed adverse events during the entire study period. There is no statistically significant difference found at baseline S mutans and L acidophilus count ($p > 0.05$).

- *S Mutans and L Acidophilus Bacterial Count*

Both S mutans and L acidophilus mean bacterial counts reduced comparing to the baseline (Figure 2.2 and 2.3) and found to be statistically significant difference found at 14^{th} day S mutans and L acidophilus count ($p = 0.000$ and $p = 0.001$ respectively) (Table2).

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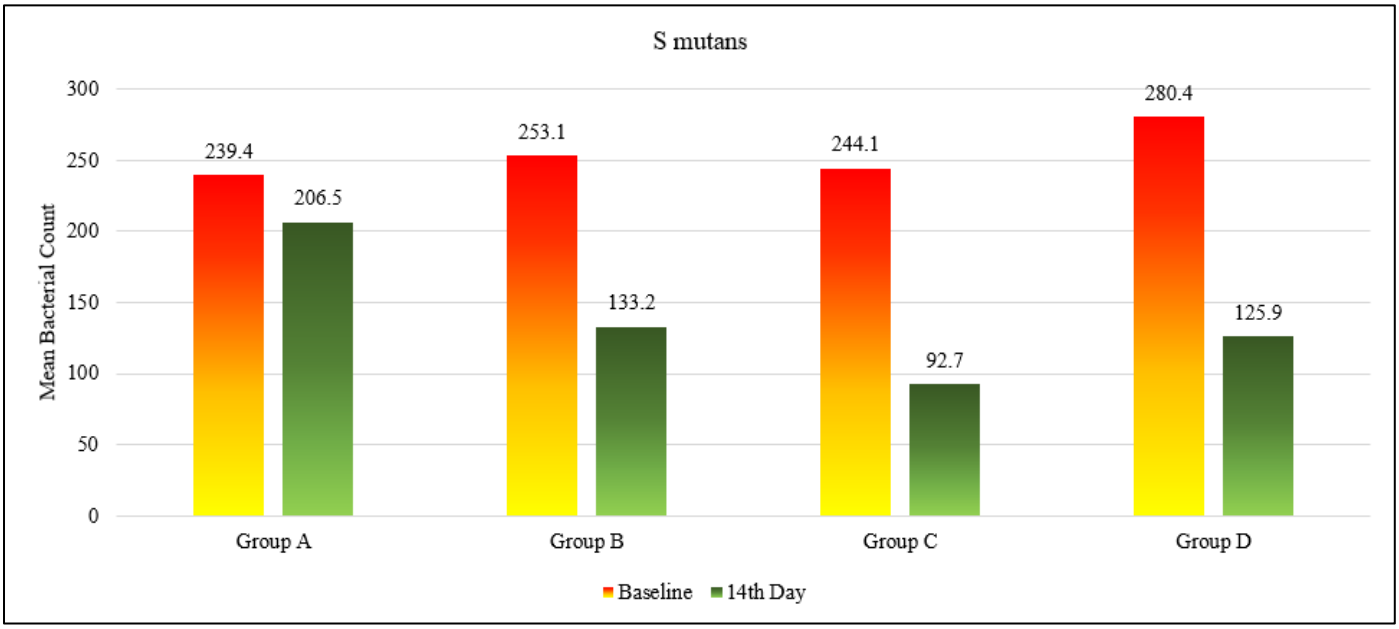

Fig 2 Intragroup Comparison of S. Mutans before and after Intervention

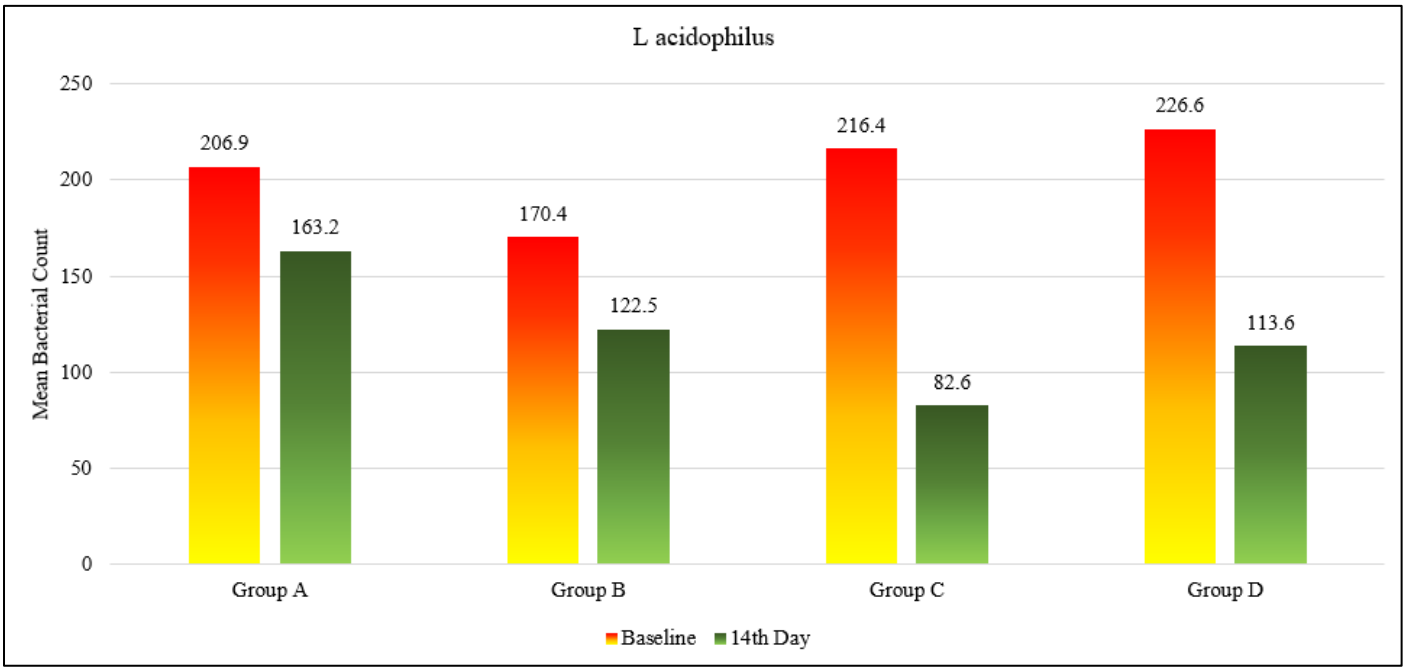

Fig 3 Intragroup Comparison of L Acidophilus before and after Intervention

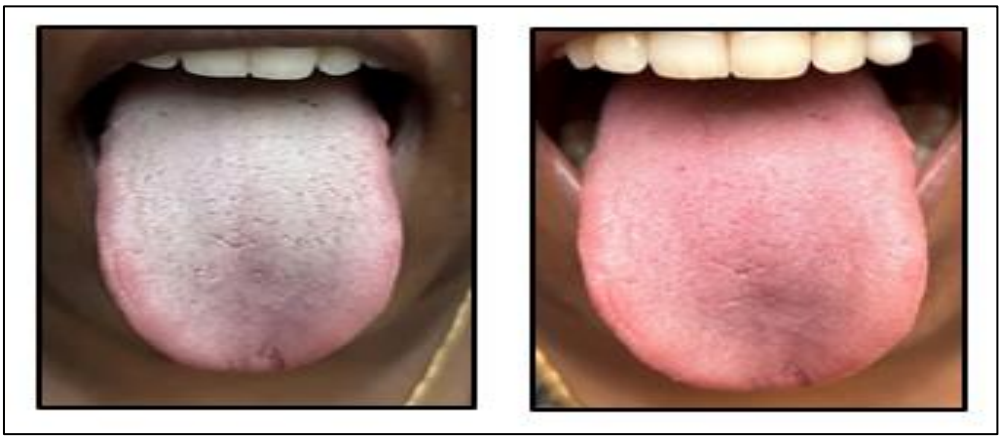

Fig 4 Before and after Benzalkonium Chloride Gel

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➢ *Winkel Tongue Coating Index*

The tongue coating was reduced in Group C participants who were using tongue brush along with benzalkonium chloride gel (Figure 4). All the groups were statistically significant in reducing the tongue coating on dorsal tongue surface, except for Group A participants ($p > 0.05$) (Table 3).

Table 2 Intragroup Comparison of Bacterial Count before and after Intervention

GROUPS	Bacteria	n	Baseline Mean (SD)	14th Day Mean (SD)	p Value
GROUP A (TONGUE BRUSH)	*S mutans*	15	239.40 (±39.6)	206.50 (±36.8)	0.008*
	L acidophilus		206.9 (± 68.9)	163.2 (± 61.1)	0.003
GROUP B (TONGUE BRUSH + CLOHEX ADS MW)	*S mutans*	15	253.10 (±64.2)	133.20 (±61.3)	0.000*
	L acidophilus		170.4 (± 41.5)	122.5 (± 31.6)	0.012*
GROUP C (TONGUE BRUSH + BENZALKONIUM CHLORIDE GEL)	*S mutans*	15	244.10 (±71.6)	92.70 (±14.2)	0.000*
	L acidophilus		216.4 (± 44.9)	82.6 (± 12.4)	0.000*
GROUP D (TONGUE BRUSH + HEXIGEL	*S mutans*	15	280.40 (±45.3)	125.90 (±27.5)	0.000*
	L acidophilus		226.6 (± 44.2)	113.6 (± 15.4)	0.000*

* - p value less than 0.05 is statistically significant

Table 3 Intragroup comparison of tongue coating before and after intervention

Groups	Interval	n	Mean Rank (IQR)	p Value
GROUP A Tongue Brush	Baseline	15	23.90 (2.50)	0.079
	14th Day		23.85 (2.00)	
GROUP B Clohex Mouthwash + Tongue Brush	Baseline	15	19.35 (2.00)	0.014*
	14th Day		14.75 (1.00)	
GROUP C Benzalkonium Chloride Gel+ Tongue Brush	Baseline	15	23.50 (3.00)	0.007*
	14th Day		12.50 (1.00)	
GROUP D Hexigel + Tongue Brush	Baseline	15	20.75 (2.00)	0.008*
	14th Day		20.30 (1.00)	

* - p value less than 0.05 is statistically significant

IV. DISCUSSION

Tongue coating is a natural phenomenon in the oral cavity. The tongue coating is characterized by the white appearance of the tongue caused by residue, white blood cells, bacteria, fungi, and food residue removed in between the papillae.[14] While a thin coating is acceptable, It might act as a protective barrier on the tongue's surface, shielding it from irritation caused by certain foods or acidic beverages.[15] Whereas, a thick or persistent coating might warrant improved oral hygiene practices or a visit to the dentist to rule out any underlying issues. Brushing and scraping the tongue can be effective management strategies, but a holistic approach regarding oral health and potential connections to overall well-being is crucial. Tongue coating, along with dental plaque, serves as a scaffold for the growth of oral bacteria and is thought to be one of the causes of aspiration pneumonia in elderly patients.[16]

Oral bacteria are decreased by practicing better oral hygiene, including scrapping the tongue using a toothbrush or tongue scraper to remove food particles, cells, and germs trapped between the tongue papilla.[17] It has been stated that using a regular toothbrush for tongue cleaning is inferior for removing debris and organisms from the tongue compared with using a scraping debridement tool.[18]

However contraindicating results were seen in a study conducted by Laleman et al who reported that there are no significant differences in using toothbrushes and tongue scrappers in cleaning the dorsal surface and did not influence the bacterial load in the saliva or on the tongue dorsum among periodontitis patients.[19] Dwivedi et al reported that metal and plastic tongue scrapers showed a significant reduction of the anaerobic bacterial count on the tongue, compared to the head of the toothbrush.[20]

Streptococcus mutans and *Lactobacillus acidophilus* are causative bacteria for caries and are evident in the formation of tongue coating. In the present study, there was a gradual reduction in the Streptococcus mutans count from baseline to the 14th day after tongue brushing which is similar to the study conducted by Gondhalekar et al. [21]

Bordas et al reported that while mechanical tongue cleaning with or without chemical intervention can reduce bacterial load on the tongue, this effect is transient, and regular tongue cleaning is required to provide a long-lasting (overnight) reduction in bacterial numbers. Compared to the Clohex ADS mouthwash, Hexigel, and Benzalkonium Chloride gel shows an increased reduction in the mean bacterial count, this is due to the thixotropic property of Gels have a thicker consistency that allows contact time on the applied surface for a longer duration, whereas designed to be swished around the mouth for a short time. Similarly, Quirynen reported that brushing the dorsum of the tongue with chlorhexidine gel significantly reduced bacterial count compared to rinsing the tongue with Clohex ADS mouthwash.[22]

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The inter-group comparison of Bacterial count in all four groups showed a statistically significant difference at the 14th day. The mean bacterial count in Group B and Group D of the tongue is decreased by the foaming reaction during the breakdown, physical removal, and mechanical washing of the tongue deposits, and the bactericidal action of Chlorhexidine, similar to the study conducted by Keceli et al in reducing the halitosis and bacterial load.[23]

In this study, it was found that after 2 weeks of tongue cleaning with a Tongue brush with benzalkonium chloride gel significantly reduced the amount of visible plaque on the dorsum of the tongue according to Winkel et al tongue coating Index was significant less after 2 weeks of tongue cleaning, similar to the study conducted by Keceli et al [23]; Except for Group A, where it could be due to the combined effect of using chemical measures. Additionally, the study participants experienced a cleaner tongue.

The mean value of tongue coating obtained by WTCI showed a reduction with all three aids although a highly significant reduction was seen with plastic tongue scraper. An approximately 55% reduction of tongue coating was seen with plastic tongue scraper in the post-intervention results. The rough surface of the plastic scraper helps to remove the tongue coating by penetrating deep into the muscular folds of the tongue. Funahara et al concluded additive effects of mechanical and chemical tongue cleaning aids showed a higher reduction as compared to the present study i.e. 74% reduction was seen on tongue coating.[24]

Similarly, Shimizu et al., revealed that tongue brushing can increase taste recognition in old age adults by eliminating the thick bacterial coating present on the tongue. Similarly, as the WTCI rises, more bacteria cling to the tongue's surface.[25]

- *Strength*

The present study was conducted in a controlled manner. Baseline parameters were standardized and compared with a gold-standard drug, thereby reducing the impact of tongue coating and bacterial load on the dorsal tongue surface. It also has better palatability than Hexigel.

V. LIMITATIONS

A smaller sample size and a shorter follow-up period of 14 days, difficulty in generalizing the results, and only healthy volunteers have participated but the oral microflora for patients having various systemic diseases and other conditions has not been evaluated, Oral malodor and moisture levels in the tongue have to be assessed. Second, to consider this study's clinical applicability, several issues must be addressed, including the cost-effectiveness, the addition of coloring agents to improve acceptability, and its long-term effectiveness.

VI. FUTURE RECOMMENDATIONS

Further studies focus on large sample sizes, longitudinal studies, and cross-over trials to evaluate the effect of tongue brushes with various antibacterial agents in reducing the bacterial count and tongue coating on the dorsal tongue surface.

VII. CONCLUSION

Benzalkonium Chloride gel showed similar antibacterial as comparing to CHX mouthwash and CHX gel. A Combined effect is seen when the chemical measures are used along with the mechanical method of removing the tongue coating by a tongue brush. Therefore, they can resolve tongue coating, thereby leading to improved oral hygiene.

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➢ *Supplementary*

Table 1 Intergroup Comparison of Bacterial Count before and after Intervention

BACTERIA	GROUPS	n	Baseline Mean (SD)	p Value	14th Day Mean (SD)	p Value
S mutans	GROUP A Tongue Brush	15	239.4 (± 39.6)	0.372	206.5 (± 36.8)	0.000*
	GROUP B Clohex Mouthwash + Tongue Brush	15	253.1 (± 61.3)		133.2 (± 64.2)	
	GROUP C Benzalkonium Chloride Gel + Tongue Brush	15	244.1(± 71.6)		92.7 (±14.2)	
	GROUP D Hexigel + Tongue Brush	15	280.4 (± 45.3)		125.9 (±27.5)	
L acidophilus	GROUP A Tongue Brush	15	206.9 (± 21.7)	0.078	163.2 (61.1)	0.001*
	GROUP B Clohex Mouthwash + Tongue Brush	15	170.4 (± 10.0)		122.5 (±41.5)	
	GROUP C Benzalkonium Chloride Gel+ Tongue Brush	15	216.4 (± 14.2)		82.6 (±12.4)	
	GROUP D Hexigel + Tongue Brush	15	226.6 (± 13.9)		113.6 (±15.4)	

➢ *Supplementary*

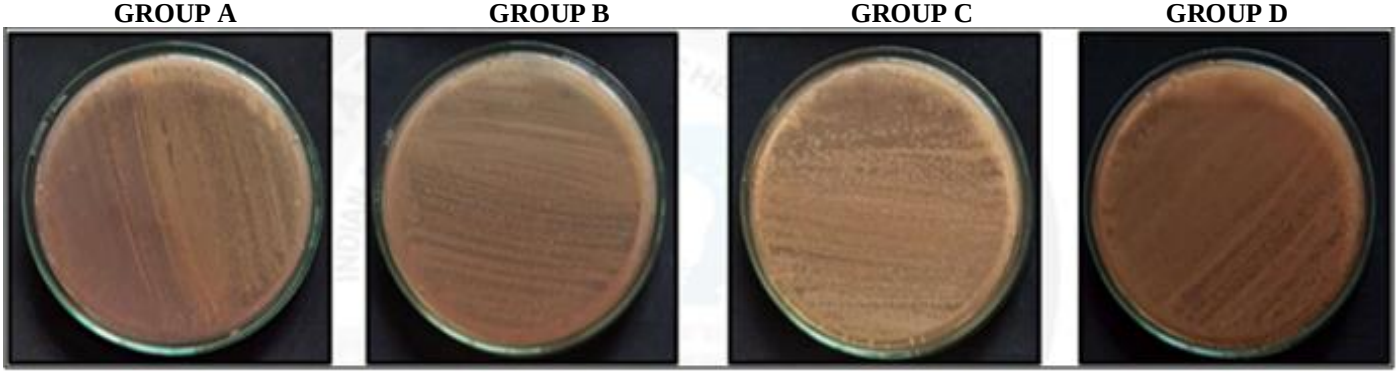
Table 2: Intergroup Comparison of Tongue Coating before and after Intervention

Groups	N	Baseline Mean Rank (IQR)	Sig	14th Day Mean Rank (IQR)	Sig
GROUP A Tongue Brush	15	23.00 (2.5)	0.230	23.85 (2.0)	0.003*
GROUP B Clohex Mouthwash + Tongue Brush	15	19.35 (2.0)		14.75 (1.0)	
GROUP C Benzalkonium Chloride Gel+ Tongue Brush	15	23.50 (3.0)		12.50 (1.0)	
GROUP D Hexigel + Tongue Brush	15	20.75 (2.0)		20.30 (1.0)	

➢ *Supplementary*

- *Before*

GROUP A **GROUP B** **GROUP C** **GROUP D**



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- *After*

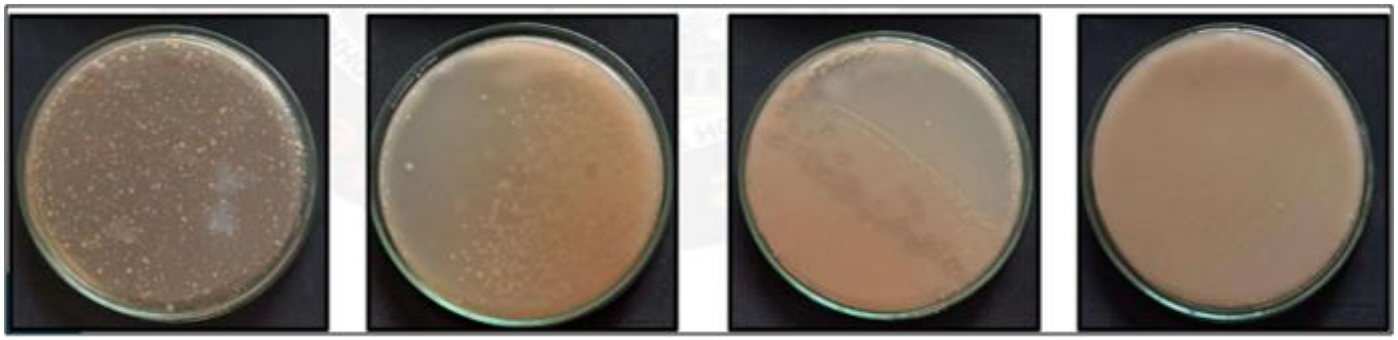
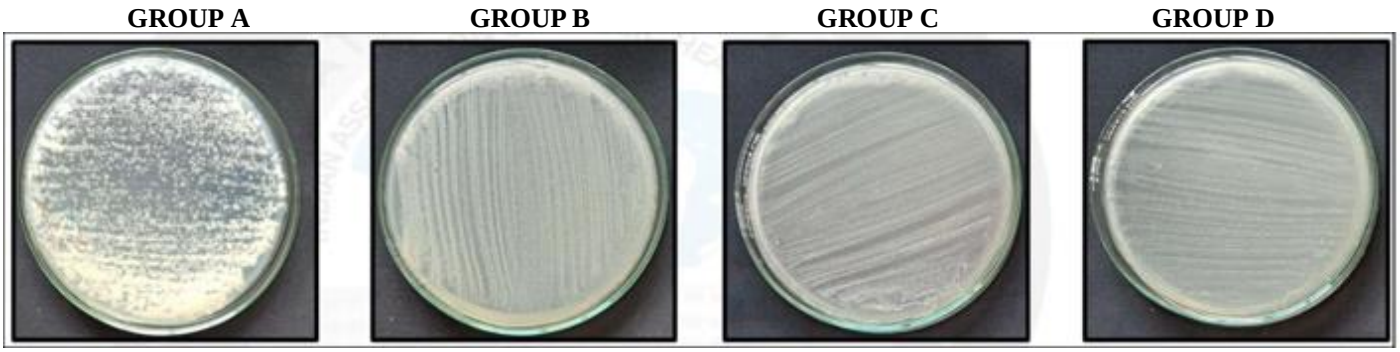


Fig 1 Before and After Intervention - Microbial Culture plates for S mutans

➢ *Supplementary*

- *Before*



- *After*

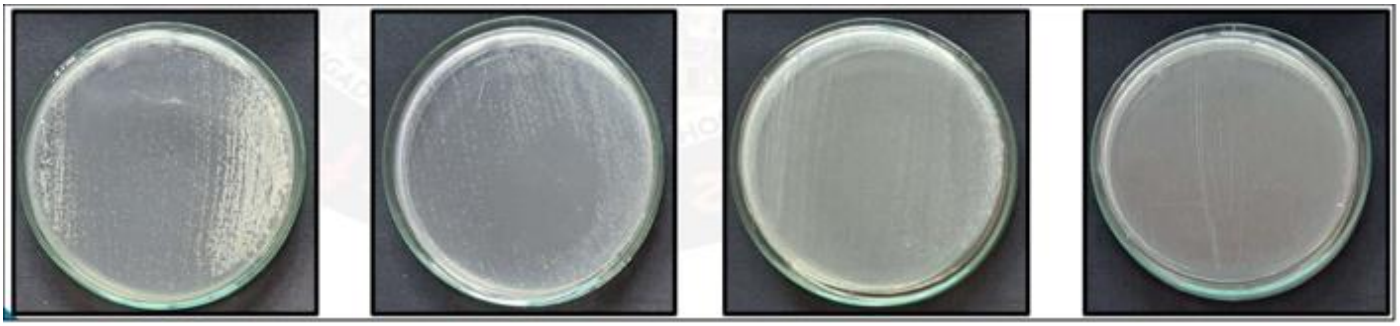


Fig 2 Before and After Intervention - Microbial Culture Plates L acidophilus.