Original Article

Comparing the Effectiveness of Probiotic, Green Tea, and Chlorhexidine- and Fluoride-containing Dentifrices on Oral Microbial Flora: A Double-blind, Randomized Clinical Trial

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Abstract

Introduction: Oral cavity harbors wide variety of microorganisms; these are considered crucial for the dental caries initiation and progression. Plaque-induced caries is a local disease; therefore, dentifrices are the most ideal vehicle for the daily delivery of antibacterial agents. In recent years, alternatives to fluorides such as green tea, probiotic, and chlorhexidine (CHX) toothpastes have been proposed to possess antiplaque and anticariogenic properties. **Aim:** To compare the effectiveness of probiotic, green tea, and CHX- and fluoride-containing dentifrices on oral microbial flora. **Materials and Methods:** A double-blinded, parallel group, randomized controlled clinical trial was conducted among healthy adults. Fifty-two individuals were randomly allocated to four groups (n = 13): Group I – green tea dentifrice, Group II – fluoridated dentifrice, Group III – CHX dentifrice, and Group IV – probiotic dentifrice. Plaque and saliva samples were evaluated for *Streptococcus mutans* and *Lactobacillus* at baseline and 15th and 30th days of follow-up. Paired t-test and one-way ANOVA were used to compare the mean differences of plaque and salivary *S. mutans* counts at two and three time periods. Wilcoxon signed-rank and Kruskal–Wallis tests were used to compare the mean *Lactobacillus* count in plaque and saliva samples at two and three time periods, respectively. **Results:** The mean *S. mutans* and *Lactobacillus* counts in plaque and saliva samples were significantly reduced by all the treatment groups at the 30th day of follow-up. However, Group III showed the highest reduction and was found to be statistically significant ($P < 0.05$). **Conclusion:** All the four groups exhibited antimicrobial activity by bringing about a significant reduction in the mean *S. mutans* and *Lactobacillus* colony counts at the 30th day of follow-up. Among all the preventive modalities, Group III (CHX dentifrice) showed better results compared to other groups.

Keywords: *Chlorhexidine dentifrice, green tea dentifrice, Lactobacillus, probiotic dentifrice, Streptococcus mutans*

Introduction

Dental caries is a major public health and continuing problem worldwide. Among all the causes of disability-adjusted life years evaluated in the Global Burden of Disease 2010 Study, the global prevalence of untreated caries was the highest, with no decreasing trends between 1990 and 2010, and its global burden is ranked 80th.[1]

Over 400 species of microbes inhabit as commensals in the oral cavity of a healthy adult.[2] An aberration to this ecology due to dietary habits, improper oral hygiene, or systemic factors leads to an increased cariogenic microorganisms.[3] Cariogenic microorganisms such as *Streptococcus mutans* and *Lactobacillus acidophilus* are the primary causative microorganisms for the development of dental caries. These cariogenic microorganisms encourage the accumulation and adherence of plaque biofilm by metabolizing sucrose into sticky glucan. The microorganisms in dental plaque degrade the dietary carbohydrates producing lactic acid leading to localized demineralization and the eventual formation of dental caries.[4]

Plaque-induced caries is a local disease; therefore, the local use of antimicrobial agents is more efficient than their systemic use.[5] Numerous strategies and measures have been adapted to eliminate plaque and reduce the bacterial colony counts to preserve the oral health for lifetime. One among them is toothbrushing. As toothbrushing is considered to be the most common oral hygiene method, dentifrices

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are the most ideal vehicle for the daily delivery of antibacterial agents. These chemotherapeutic agents should provide a preventive effect against caries and gingivitis.[6]

Toothbrushing with fluoridated toothpaste is the most widespread form of fluoride usage.[7,8] Even though fluoridated toothpastes were considered to be gold standard for the prevention of dental caries, concern has been expressed that dental fluorosis, enamel defects caused by young children chronically ingesting excessive amounts of fluoride during the period of tooth formation (up to the age of 6 years), is increasing in both fluoridated and nonfluoridated communities, and the early use of fluoride toothpastes by young children may be an important risk factor.[9,10] The side effects encountered with the use of fluoridated toothpaste formulations has led to the search for novel and safe alternatives.

In recent years, alternatives to fluorides such as green tea, probiotic, and chlorhexidine (CHX) toothpastes have been proposed to possess antiplaque and anticariogenic properties. Green tea is one such natural alternative, which possesses anticariogenic activity through a direct bactericidal effect against cariogenic microorganisms and indirectly by the prevention of bacterial adherence to teeth.[11] Several studies have indicated that bioactive components of green tea can influence the process of caries formation through several different mechanisms: they may inhibit proliferation of the streptococcal agent, interfere with the process of bacterial adhesion to tooth enamel, and act as inhibitors of glucosyltransferase and amylase.[11-14]

One of the novel strategies for the prevention of dental caries is by manipulation of resident oral microorganism by ingestion of probiotic organisms.[15] The topical application of probiotic toothpaste caused significant decreases in the *S. mutans* levels in the plaque around the brackets of orthodontic patients.[16] Hence, probiotic dentifrices are suitable for all age groups and considered as an ideal vehicle for the replacement of cariogenic bacteria by nonpathogenic bacteria to prevent dental caries.

CHX is a cationic antiseptic with action against a wide array of bacteria including Gram-positive and Gram-negative bacteria, dermatophytes, and some lipophilic viruses. CHX acts on the bacterial cell membrane by changing its structure. As a result, osmotic equilibrium is lost, the membrane extrudes, vesicles are formed, and the cytoplasm precipitates.[17] The superiority of this agent as compared to other chemical derivatives is mainly due to its substantivity, which in turn prolongs its antibacterial action and prevents dental caries.

Clinical trials conducted in the recent years have evaluated the beneficial effects of green tea, probiotic, and CHX by means of various delivery systems and vehicles such as mouthrinse, chewing gum, tablets, lozenges, and powder. Therefore, there are only limited data, and very few studies have explored the clinical effectiveness of green tea, probiotic, and CHX dentifrices, since dentifrices are considered to be the most ideal vehicle for the daily delivery of antimicrobial agents.

Hence, the present study was conducted with the aim to compare the effectiveness of probiotic, green tea, and CHX- and fluoride-containing dentifrices on oral microbial flora.

Materials and Methods

Study design

It is a double-blinded, parallel group, randomized controlled clinical trial.

Sample size determination

The sample size was calculated based on the study by Burton *et al*.[18] using *a priori* by G*Power Software Version 3.0.1.0 (Franz Faul, Universitat Kiel, Germany). The minimum sample size of each group was calculated, following these input conditions: power of 0.95 and $P \leq 0.05$ and the sample size arrived was 13 per group.

Ethical clearance

Before the start of the study, ethical clearance was obtained from the Institutional Ethics Committee (SRB/SDMDS12ORT16). The study was submitted to the Clinical Trials Registry-India, and the acknowledgment number is REF/2015/10/010000 and the registration number is CTRI/2016/10/007404.

Eligibility criteria

Apparently healthy individuals without any known history of systemic illness above 18–25 years of age having a DMFT score of <3 and with mild-to-moderate gingivitis were included in the study. Participants with a positive history of usage of antimicrobial therapy and routine use of oral antiseptics in the previous 3 months and with a history of allergic or idiosyncratic reactions to product ingredients, and those who are undergoing orthodontic treatment and subjects who are allergic to lactose or fermented milk products were excluded from the study.

Randomization

Sequence generation

Computer-generated block randomization with a block size of four was used to generate the assignment schedule well in advance by a third person who was not related to the study. The investigator was blinded to the sequencing of the block and allocation of the groups. Fifty-two participants were randomly allocated to four groups ($n = 13$): Group I – green tea dentifrice, Group II – fluoridated dentifrice, Group III – CHX dentifrice, and Group IV – probiotic dentifrice [Table 1 and Figure 1].

Allocation concealment

Sequentially numbered, opaque, sealed envelopes method was implemented for allocation concealment, which conceals the sequence until interventions were assigned. Patients were

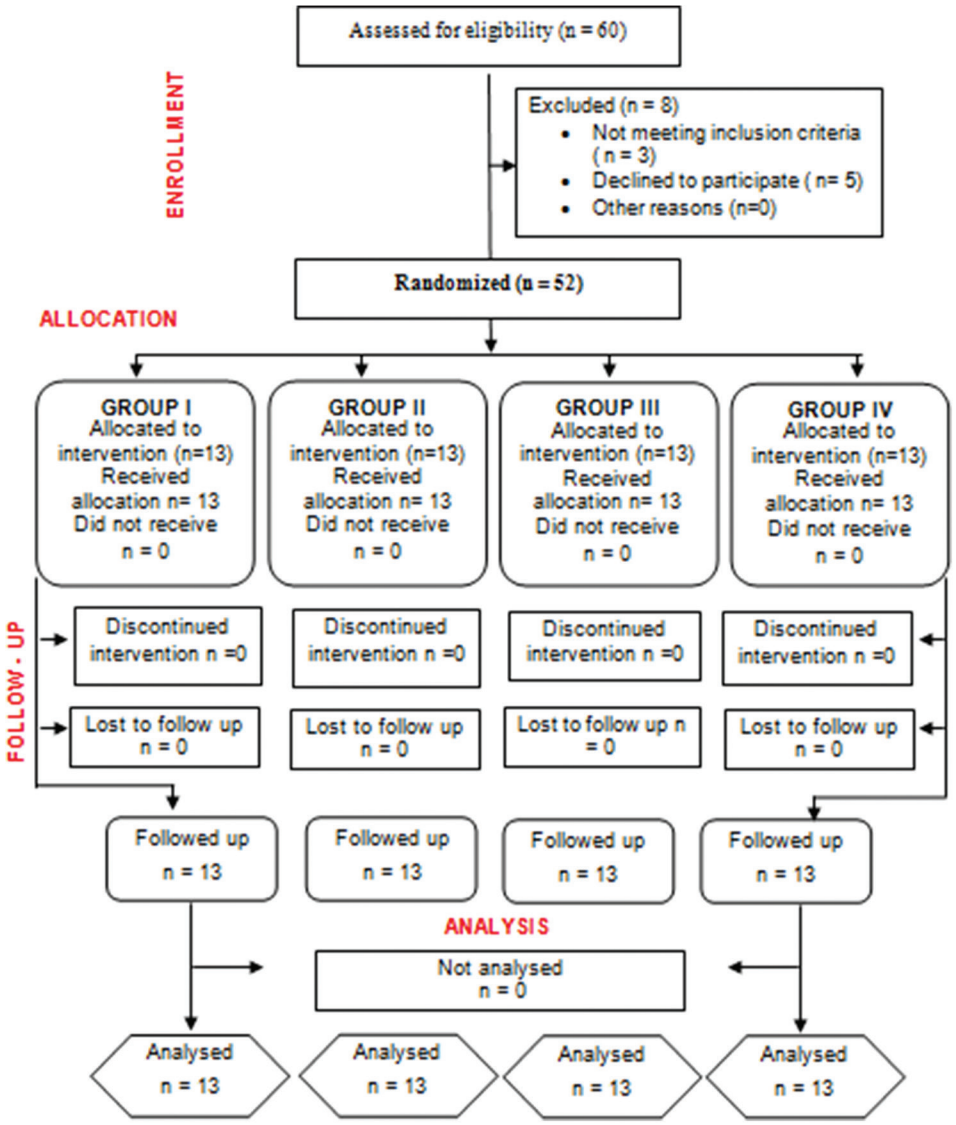

Figure 1: Participants' flowchart

Table 1: Tested products and their composition

Group	Products	Composition
I	Splat Green Tea fluoride free, strengthening toothpaste	Dentifrice containing *Camellia sinensis* leaf extract
II	Colgate Total Advanced Health Toothpaste	Dentifrice containing 1000 ppm of sodium fluoride
III	Curasept 0.12% Chlorhexidine Toothpaste	Dentifrice containing 0.12% Chlorhexidine
IV	GD Probiotic Toothpaste	Dentifrice containing bacteriocin

assigned their study numbers as they sequentially entered into the study. Based on the group assigned, respective treatment was carried out as described in the procedure.

Blinding

Although the investigator knows about the study design and dentifrices that were used in the study, investigator is unaware about which dentifrice has been assigned to each sample. Therefore, both the investigator and microbiologist were blinded in the study.

Study procedure

- Step 1: Obtaining preoperative details and informed consent from the study participants: Before the treatment, a careful medical and dental history was taken. Preoperative data for each patient were recorded in the predesigned pro forma which includes age, gender, and address. The study design was explained to the qualifying patients, and informed consent was obtained from the voluntary patients who were willing to participate in the study
- Step 2: Method of collection of saliva sample: The study participants were instructed not to eat or drink except water and not to perform physical exercise for at least 1 h before the collection to standardize the

participants. The participants were seated comfortably on the dental chair and instructed to expectorate 1.5 ml of unstimulated saliva in a 5 ml plastic sterile container over 10 min during 9–10 am of college hours

- Step 3: Application of plaque-disclosing solution: Plaque test is generously applied to the surfaces of the teeth with the help of applicator brush. The study participants were instructed to rinse the mouth
- Step 4: Evaluation of plaque under polymerization blue light: The surface of the teeth is illuminated with a polymerization blue light. Any areas affected by plaque appear brightly fluorescent. The teeth appear blue, and the gingival tissues appear dark blue [Figure 2]
- Step 5: Method of collection of plaque sample: Pooled plaque samples were collected from the buccal surfaces of clinically sound upper first molar region with a sterilized surface scaler. The collected plaque samples were transferred to a test tube containing 1 ml of sterile phosphate-buffered saline and transported to the laboratory for microbial assessment
- Step 6: Oral prophylaxis: A complete oral prophylaxis was performed for all the participants to standardize
- Step 7: Oral hygiene instructions and toothbrushing technique: A standardized toothbrush and the toothpastes were allocated according to the group. Oral hygiene instructions with an emphasize on the appropriate brushing technique were given
- Step 8: Microbial evaluation of plaque and saliva specimen
  - I. Preparation of mitis salivarius agar culture plates for *S. mutans*: The mitis salivarius agar medium was prepared according to the manufacturer's instructions as follows: 90 g of agar was mixed with 1000 ml of distilled water and the mixture was boiled to ensure complete dissolution; this solution was then autoclaved at 15 lb pressure and at 121°C temperature for 15 min. After cooling to 50°C–55°C, 1 ml of 0.1% potassium tellurite was added to make the solution selective for streptococci organisms. This final mixture is poured into the culture plates
  - II. Preparation of *Lactobacillus* MRS agar culture plates for *Lactobacillus*
    - a. The *Lactobacillus* MRS agar medium was prepared according to the manufacturer's instructions as follows: 90 g of agar was mixed with 1000 ml of distilled water and the mixture was boiled to ensure complete dissolution; this solution was then autoclaved at 15 lb pressure and at 121°C temperature for 15 min.
  - III. Inoculation of plaque and saliva specimens
    - a. The collected plaque and saliva was then diluted to ten-folds with normal saline. Plaque mixture was placed in a vortex mixer to ensure uniform mixing of plaque with saline. The plaque and saliva samples were subjected to microbial analysis by taking 10 ml of the sample in 4 mm internal diameter inoculation loop and streaking on freshly prepared mitis salivarius agar and *Lactobacillus* MRS agar culture plates.
  - IV. Incubation of inoculated culture plates
    - a. The inoculated culture plates were placed in the incubator at 37°C for 24 h.
  - V. Counting bacterial colonies
    - a. Colonies of *S. mutans* appear with morphologic characteristics 0.5 mm raised convex undulated colonies of light blue color with rough margins, granular frosted glass appearance [Figure 3]. Colonies of *Lactobacillus* were characterized by small grayish-white, flat or raised, smooth, rough or intermediate [Figure 4]. Colonies were expressed as the number of colony-forming units (CFUs) per ml. The mean was counted from duplicate for each sample:

$$\text{Real bacterial number}\,(\text{CFU}/\text{ml}) = \frac{\text{Number of colonies} \times \text{dilution factor}}{\text{volume plated in ml}}$$

  - VI. By multiplying the actual colony count by 1×10^3, semiquantification of the number of colonies was done. The numbers of CFUs per milliliter was recorded in the prestructured pro forma.

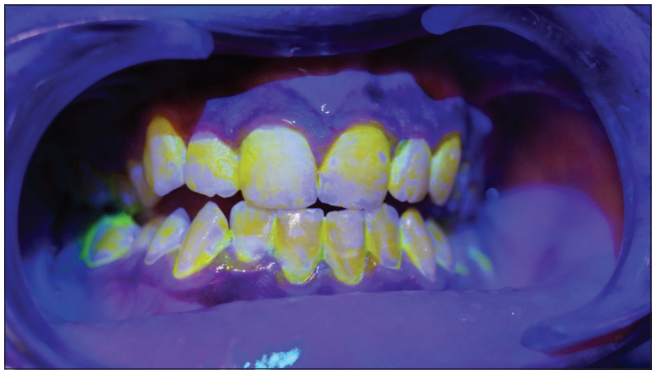

Figure 2: Disclosing the plaque with plaque test solution

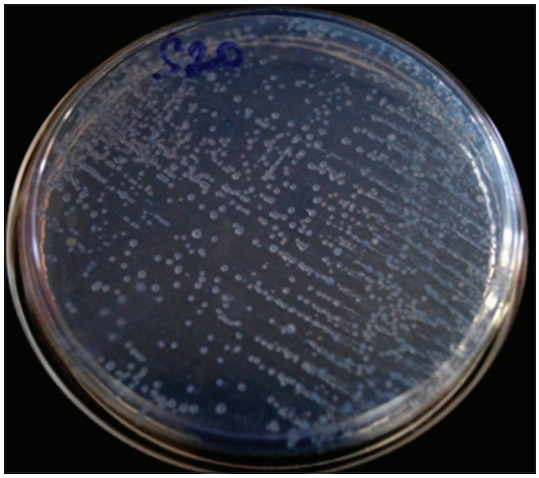

Figure 3: *Streptococcus mutans* colonies

- Step 9: Follow-up at the 15th and 30th days: The above-mentioned steps were repeated at the 15th and 30th days of follow-up.

Outcome measure

The investigator evaluated the plaque and saliva samples for *S. mutans* and *Lactobacillus* after the use of tested products at baseline and the 15th and 30th days and compared the effects of four dentifrices to determine the percentage reduction in organisms and between these groups.

Statistical analysis

Data were entered into Microsoft Excel spreadsheet and were analyzed using IBM SPSS software version 20.0 (Armonk, NY: IBM. Corp., USA). Numerical data were presented as mean and standard deviation values. For test, $P < 0.05$ was considered statistically significant. Shapiro–Wilk test was used to test the normality of the dataset. Paired *t*-test was used to compare the mean differences of plaque and salivary *S. mutans* colony counts at two time points. One-way ANOVA and *post hoc* Tukey's test was used to compare the mean differences of plaque and salivary *S. mutans* colony counts at three time points. Wilcoxon signed-rank test was used to compare the mean *Lactobacillus* count in plaque and saliva at two time points. Kruskal–Wallis test was used to compare the mean *Lactobacillus* count in plaque and saliva at three time points.

Results

Figure 5 depicts the percentage reduction of *S. mutans* count (plaque) (CFU/ml) of Groups I, II, III, and IV at two time points. All the four groups showed a percentage reduction of *S. mutans* count (plaque) (CFU/ml) from baseline to 15th day and from baseline to 30th day, while during 15th to 30th days of follow-up, there was no reduction in Groups I and IV, which showed a negative value of − 24.49 and − 15.18, respectively, but Group II and Group III showed a reduction of 12.57 and 35.2, respectively. However, percentage reduction of *S. mutans* count (plaque) of Group III was found to be highest among all the four groups at two-point comparison from baseline to 30th day (60.6). Both Group II and Group III showed a statistically significant (paired "*t*" test) difference in the mean *S. mutans* count in plaque at two-point comparison. However, the mean difference in Group III from baseline to 30th day was found to be more comparable to Group II, which signifies Group III to be superior to Group II. Table 2 shows the comparison of mean *S. mutans* count in plaque of Groups I, II, III, and IV at three time points using one-way ANOVA. There was no statistically significant difference among all the groups at baseline and the 15th day of follow-up. However, at the 30th day of follow–up, there was a significant difference among all the groups. Group III showed the highest reduction in the mean *S. mutans* count in plaque at the 15th (47,846.1 ± 30,818.9) and 30th (28,846.1 ± 16,237.0) days from baseline count (84,000.0 ± 59,136.8), and *post hoc* Tukey's analysis showed a significant difference in mean *S. mutans* count in plaque from baseline to 30th day, which was found to

Table 2: Comparison of mean *Streptococcus mutans* count in plaque (colony-forming units/ml) of Groups I, II, III, and IV at three time points

Time points	*n*	Mean *Streptococcus mutans* count in plaque			
		Groups	Mean±SD	*F*	*P*
Baseline	13	I	58,538.4±45,152.7	2.557	>0.05
		II	36,000.0±15,465.0		
		III	84,000.0±59,136.8		
		IV	58,923.0±45,233.5		
15th day	13	I	37,307.6±26,575.0	1.739	>0.05
		II	25,615.3±12,052.2		
		III	47,846.1±30,818.9		
		IV	39,307.6±26,568.7		
30th day	13	I	44,307.6±28,633.8	3.447	<0.05
		II	21,615.3±9069.5		
		III	28,846.1±16,237.0		
		IV	45,307.6±29,923.1		

One-way ANOVA ($P<0.05$). SD: Standard deviation

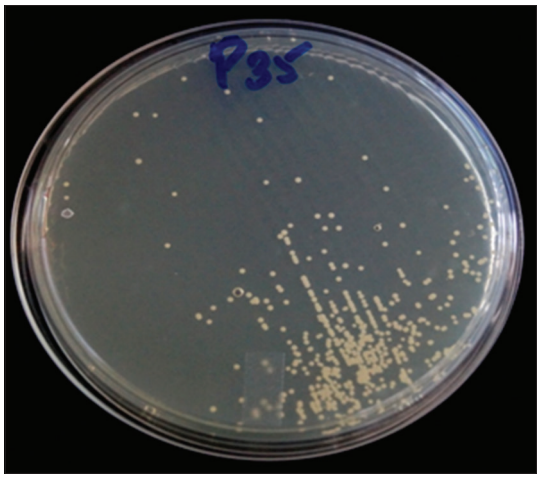

Figure 4: *Lactobacillus* colonies

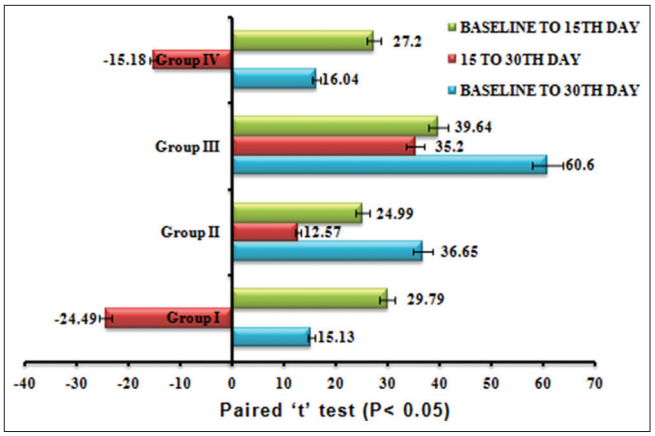

Figure 5: Percentage reduction of plaque *Streptococcus mutans* count (CFU/ml) of Groups I, II, III, and IV at two time points

be statistically significant ($P < 0.05$). Table 3 shows the comparison of mean *Lactobacillus* count (CFU/ml) of Groups I, II, III, and IV in plaque at two time points using Wilcoxon signed-rank test. Among all the groups, Group III showed a statistically significant difference ($P < 0.05$) at two-point comparison. Table 4 shows the comparison of mean *Lactobacillus* count (CFU/ml) of Groups I, II, III, and IV in plaque at three time points using Kruskal–Wallis test. Among all the groups, Group III showed a statistically significant difference ($P < 0.05$) at the 15th and 30th days of follow-up. Figure 6 depicts the percentage reduction of salivary *S. mutans* count (CFU/ml) of Groups I, II, III, and IV at two time points. All the four groups showed a percentage reduction of salivary *S. mutans* count (CFU/ml) from baseline to 15th day, from 15th to 30th day, and from baseline to 30th day. However, the percentage reduction of salivary *S. mutans* count of Group III was found to be highest among all the four groups at two-point comparison from baseline to 30th day (52.9). Both Group II and Group III showed a statistically significant (paired "*t*" test) difference in the mean *S. mutans* count in saliva at two-point comparison. However, Group III showed a highly significant difference ($P < 0.01$). Table 5 shows the comparison of salivary *S. mutans* count (CFU/ml) of Groups I, II, III, and IV at three time points using one-way ANOVA. There was no statistically significant difference in the mean salivary *S. mutans* count (CFU/ml) among all the groups at baseline and the 15th and 30th days of follow-up and *post hoc* Tukey's analysis showed a significant reduction in the mean salivary *S. mutans* count in Group III compared to other groups. Table 6 shows the comparison of mean *Lactobacillus* count (CFU/ml) of Groups I, II, III, and IV in saliva at two time points using Wilcoxon signed-rank test. Among all the groups, Group III showed a statistically significant difference ($P < 0.05$) at two-point comparison. Table 7 shows the comparison of mean *Lactobacillus* count (CFU/ml) of Groups I, II, III, and IV in saliva at three time points using Kruskal–Wallis test. Among all

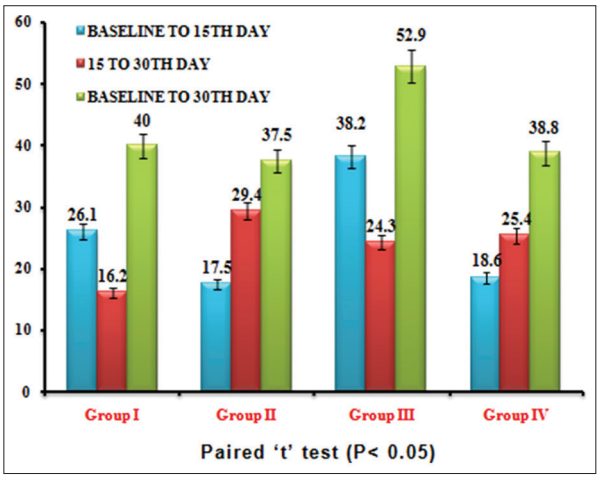

Figure 6: Percentage reduction of salivary *Streptococcus mutans* count (CFU/ml) of Groups I, II, III, and IV at two time points

Table 3: Comparison of mean *Lactobacillus* count (colony-forming units/ml) of Groups I, II, III, and IV in plaque at two time points

Groups	*n*	Mean *Lactobacillus* count in plaque		
		Time points	*Z*	*P*
I	13	Baseline to 15th day	1.620	>0.05
		15th to 30th day	1.892	>0.05
		Baseline to 30th day	2.366	>0.05
II	13	Baseline to 15th day	1.84	>0.05
		15th to 30th day	2.12	>0.05
		Baseline to 30th day	2.49	<0.05
III	13	Baseline to 15th day	2.67	<0.05
		15th to 30th day	2.95	<0.05
		Baseline to 30th day	3.07	<0.05
IV	13	Baseline to 15th day	-1.02	>0.05
		15th to 30th day	0.797	>0.05
		Baseline to 30th day	-0.712	>0.05

Wilcoxon signed-rank test ($P<0.05$)

Table 4: Comparison of mean *Lactobacillus* count (colony-forming units/ml) of Groups I, II, III, and IV in plaque at three time points

Groups	*n*	Mean *Lactobacillus* count in plaque			
		Time points	Mean±SD	χ^2	*P*
I	13	Baseline	9538.4±12,066.5	1.064	>0.05
		15th day	7461.5±10,162.1		
		30th day	3461.5±4135.5		
II	13	Baseline	7230.7±10,771.5	2.261	>0.05
		15th day	5461.5±9412.8		
		30th day	3692.3±7739.3		
III	13	Baseline	9538.4±14,523.6	13.70	<0.05
		15th day	48,461.1±8414.7		
		30th day	1538.4±3710.6		
IV	13	Baseline	7615.3±12,790.2	0.288	>0.05
		15th day	8076.9±8460.3		
		30th day	7692.3±8024.8		

Kruskal-Wallis test ($P<0.05$). SD: Standard deviation

Table 5: Comparison of salivary *Streptococcus mutans* count (colony-forming units/ml) of Groups I, II, III, and IV at three time points

Time points	*n*	Mean *Streptococcus mutans* count in saliva			
		Groups	Mean±SD	*F*	*P*
Baseline	13	I	240,076.9±115,195.8	2.276	>0.05
		II	161,538.4±85,728.8		
		III	243,692.3±109,960.1		
		IV	269,615.3±130,751.3		
15th day	13	I	181,307.6±103,627.0	2.552	>0.05
		II	127,692.3±67,661.3		
		III	150,000±68,934.7		
		IV	22,0461.5±113,854.1		
30th day	13	I	152,461.5±95,213.4	1.864	>0.05
		II	94,538.4±52,103.4		
		III	119,461.5±69,726.3		
		IV	153,307.6±76,334.1		

One-way ANOVA ($P<0.05$). SD: Standard deviation

Table 6: Comparison of mean *Lactobacillus* count (colony-forming units/ml) of Groups I, II, III, and IV in saliva at two time points

Groups	*n*	Mean *Lactobacillus* count in saliva		
		Time points	*Z*	*P*
I	13	Baseline to 15th day	1.96	>0.05
		15th to 30th day	2.75	<0.05
		Baseline to 30th day	2.58	<0.05
II	13	Baseline to 15th day	2.27	>0.05
		15th to 30th day	2.48	<0.05
		Baseline to 30th day	2.47	<0.05
III	13	Baseline to 15th day	2.65	<0.05
		15th to 30th day	2.93	<0.05
		Baseline to 30th day	2.93	<0.05
IV	13	Baseline to 15th day	0.31	>0.05
		15th to 30th day	1.84	>0.05
		Baseline to 30th day	0.01	>0.05

Wilcoxon signed-rank test (P<0.05)

Table 7: Comparison of mean *Lactobacillus* count (colony-forming units/ml) of Groups I, II, III, and IV in saliva at three-time points

Groups	*n*	Mean *Lactobacillus* count in saliva			
		Time points	Mean±SD	χ^2	*P*
I	13	Baseline	26,692.3±17,080.1	1.78	>0.05
		15th day	21,076.9±12,127.5		
		30th day	18,461.5±10,813.6		
II	13	Baseline	25,769.2±21,366.4	2.93	>0.05
		15th day	18,923.0±14,545.6		
		30th day	12,846.1±12,555.5		
III	13	Baseline	22,538.4±26,958.6	5.80	<0.05
		15th day	14,000.0±17,785.7		
		30th day	8538.4±12,816.7		
IV	13	Baseline	27,538.4±26,371.1	0.30	>0.05
		15th day	29,538.4±26,544.3		
		30th day	25,615.3±26,020.9		

Kruskal-Wallis test (P<0.05). SD: Standard deviation

the groups, Group III showed a statistically significant difference (P < 0.05) at the 15th and 30th days of follow-up.

Discussion

Oral diseases including dental caries, periodontal diseases, and tooth loss may significantly impact a person's overall health,[19] and these diseases qualify as major health problems owing to their high prevalence and incidence in all regions of the world.[20] Among these oral diseases, dental caries continues to plague most of the world's population despite overly optimistic claims of success in the elimination of this disease.[21]

The Centers for Disease Control and Prevention suggests that everyday use of a toothbrush is essential for maintaining optimum oral health.[22] As toothbrushing is considered to be the most common oral hygiene method, dentifrices are the most ideal vehicle for the daily delivery of antibacterial agents. These chemotherapeutic agents should provide a preventive effect against caries and gingivitis.[6] It is noteworthy that toothbrushing as an isolated effect, i.e., without the therapeutic effect of fluoride, has only a limited effect on caries control.[23,24] Thus, regular toothbrushing with a fluoridated toothpaste is essential to control caries.[23-25] The side effects encountered with the use of fluoridated toothpaste formulations has led to the search for novel and safe alternatives. This necessitates the need for the study.

Saliva and plaque are two of the most common oral samples collected for detecting the clinical effectiveness of these antimicrobial agents. Saliva plays an important role in maintaining the teeth integrity by buffering acids produced by cariogenic bacteria and protecting teeth from decay. Saliva may influence the oral microflora by adsorbing to the tooth surface forming the acquired pellicle that determines which microorganisms can attach and colonize.[26] Saliva has been conventionally used as a diagnostic tool to determine individual caries activity and risk.[27,28]

Although salivary analysis may provide a general overview of the oral ecology reflecting the caries risk, dental caries is principally a biofilm-induced disease.[29] Viewing this biofilm (dental plaque) as a complex microbial ecosystem has enhanced the understanding of its role in caries development and progression.[30] Hence, both saliva and plaque samples were analyzed for *S. mutans* and *Lactobacillus* in the present study.

Traditional culture method is considered to be one of the most common methods used to quantify cariogenic bacteria in plaque and saliva. In a study, Dasanayake *et al*.[31] concluded that mitis salivarius-bacitracin (MSB) agar seems to be more sensitive in detecting streptococcus strains. Hence, in the present study, MSB selective culture medium was used for assessing the colonies of *S. mutans* and MRS agar culture medium for *Lactobacillus* colonies. In this study, the standard plate counting method was used for plaque and saliva bacterial colonies expressed in CFU/ml.

In the present study, fluorescein-based disclosing solution was used to disclose plaque due to its several advantages over other plaque-disclosing agents. Fluorescein stains only the plaque, the gums, and tongue and restorations keep their own color. In addition, fluorescein is not visible in daylight, and as a result, the use of this agent does not entail any esthetic impairment.[32] Disclosing agent was applied all over the surfaces of the teeth and scored using plaque index by Silness and Loe (1964). To detect the changes in gingival inflammation, gingival index by Loe and Silness (1967) was used in the present study.

An oral prophylaxis was carried out on all the study participants to standardize the oral hygiene levels and to ensure uniformity of oral hygiene status. Similar method

of recording was observed in other studies.[33,34] Although oral hygiene practices of the study participants were not supervised, compliance was noted by the investigator every week by observing the amount of dentifrice that was remaining.

In the present study, the participants were monitored for a period of 1 month and the assessment of salivary and plaque microbial count was done at baseline and the 15th and 30th days. This is quite similar to a study done by Patil *et al*.[35]

Commercially available dentifrices were used in the present study, which include Splat Green Tea Toothpaste (Group I) containing *Camellia sinensis* leaf extract, Colgate Total Advanced Health (Group II) containing 1000 ppm of sodium fluoride, Curasept (Group III) containing 0.12% CHX, and GD Probiotic Toothpaste (Group IV) containing bacteriocin.

The present study was a double-blinded, randomized clinical trial, wherein the investigator and statistician were not aware to which group the participants belonged to. The results of this research indicated that before any intervention, there were no significant differences in the baseline values between the groups. Hence, it was possible to make a comparison between the effectiveness of these groups on the plaque, gingival status, and plaque and salivary *S. mutans* and *Lactobacillus*. No side effects were observed during the study procedure.

Group III showed a highest reduction in the mean *S. mutans* and *Lactobacillus* count in plaque and saliva at the 15th and 30th days from baseline count. Similar results were obtained by Kulkarni and Damle,[36] in which CHX has shown highly significant reduction in the mutans streptococci count. Contrary results were reported in a study conducted by Thomas *et al*.[37] Green tea mouthrinse was found to be significantly better than CHX mouth rinse against streptococcus colony counts. CHX mouthrinse was significantly better than green tea mouthrinse with respect to *Lactobacillus* colonies and even in a study done by Ferrazzano *et al*.,[38] who reported that the green tea group showed a statistically significant reduction in colony counts of mutans streptococci and lactobacilli relative to the control group. Comparable results were obtained by Tehrani *et al*.,[39] who reported that green tea mouthrinse showed a significant reduction of colony number of salivary *S. mutans* and *Lactobacillus*, which is comparable with sodium fluoride mouthrinse. In a study done by Jothika *et al*.,[40] CHX, sodium fluoride, and probiotic mouthwashes have statistically similar and equivalent antimicrobial effects on the susceptibility of oral plaque *S. mutans*.

Thus, in the present study, all the four groups showed a reduction in the mean plaque index and gingival index score as well as the mean *S. mutans* and *Lactobacillus* colony counts in plaque and saliva. But, a significant reduction was observed in Group III and was found to be superior compared to other groups. The reason attributed to the effectiveness of Group III is mainly due to its substantivity, which, in turn, prolongs its antibacterial action and prevents dental caries.[41] Furthermore, CHX is a cationic antiseptic with action against a wide array of bacteria including Gram-positive and Gram-negative bacteria, dermatophytes, and some lipophilic viruses.[17]

Reduction observed in the green tea group is mainly due to the inhibition of proliferation of the streptococcal agent, interfere with the process of bacterial adhesion to tooth enamel, and act as inhibitors of glucosyltransferase and amylase.[11-14] The fact responsible for reduction in the fluoride group followed by CHX is mainly due to its inhibitory effect on adhesion of *S. mutans* to the tooth structure and, therefore, inhibits insoluble dextran production by the bacteria. It inhibits tooth demineralization and also remineralizes incipient carious lesions. However, due to risk of ingestion and fluoride toxicity, it is not recommended in small children.[39] Reduction observed in probiotic was found to be very less compared to other groups and its action is due to its possible probiotic impact on the oral microbiota and the biofilm-mediated disease dental caries.[42]

The findings must, for a number of reasons, be interpreted with caution. First, the sample size is less and hence further studies are recommended with larger sample size. Second, the semiquantitative nature of the microbial estimation was a limitation. Finally, the participants were dental students and were more likely to maintain a better oral hygiene compared to the general population. Studies targeting the general population or patients with specific oral health problems should be considered.

Conclusion

Within the limitations of this study, all the four groups exhibited antimicrobial and antiplaque activity by bringing about a significant reduction in the mean plaque and gingival index and the mean *S. mutans* and *Lactobacillus* colony count at the 30th day of follow-up. Among all the preventive modalities, Group III (CHX dentifrice) showed more excellent results compared to other groups.

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Conflicts of interest

There are no conflicts of interest.

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