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RESEARCH ARTICLE

Comparative Evaluation of the Plaque Inhibitory Effect of a Herbal Extract containing Fluoridated Dentifrice to a Fluoridated Dentifrice: A Clinical and Microbiological Study

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ABSTRACT

Aim: To clinically and microbiologically evaluate and compare the plaque inhibitory effect of commercially available herbal extract containing fluoridated dentifrice to that of commercially available fluoridated dentifrice in a healthy dental cohort.

Materials and methods: A single-center, double-blinded, prospective, cohort, crossover study was conducted involving 25 healthy dental student volunteers meeting inclusion criteria. Two commercially available dentifrices: (Dentifrice A: Commercially available fluoridated dentifrice; and Dentifrice B: Commercially available fluoridated dentifrice containing Bromelain, Papain, Neem, and Meswak were selected as the test products. The study was conducted in two phases of 3 weeks duration each and a washout period of 1 week. Participants were assessed at baseline and 3 weeks using Plaque index (PI) and Gingival index (GI). At 3 weeks, a supragingival plaque was collected from the lingual surface of right mandibular first molar and microbial analysis was done. Microbiological counts were expressed as colony-forming units (CFU) per sample.

Results: On intergroup comparison, it was observed that mean reduction in PI for Dentifrice B is significantly more than that of Dentifrice A (p-value: 0.000021). There was no significant difference between the mean reduction in the GI of both Dentifrice A and Dentifrice B (p-value: 0.3040). On intergroup comparison of CFUs obtained after 3 weeks, Dentifrice B showed significantly less viable CFUs than that of Dentifrice A (p-value: 9.42E-08).

Conclusion: In the current study settings, it was observed that herbal extracts in dentifrices have an additive effect with fluoride in plaque inhibition.

Keywords: Bacterial count, Bromelain, Dentifrice, Microbial activity, Papain.

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INTRODUCTION

Dental plaque is the primary etiological factor in the initiation of plaque-induced gingival inflammation and periodontal disease.[1] The role of plaque control in the maintenance of gingival health has been well established in the literature.[2] Various chemical plaque control agents supplement mechanical plaque control to reduce or prevent plaque accumulation.[3] Fluoride is the most common antiplaque agent found in dentifrices available commercially. Fluoride has been known to have antisurfactant and antiadhesive properties, which inhibit plaque formation.[4]

Herbal extracts, such as Curcumin (*Curcuma longa*), Aloe vera (Asphodelaceae), Meswak (*Salvadora persica*), and Neem (*Azadirachta indica*) have been included in the composition of commercially available dentifrices. A number of controlled clinical trials have demonstrated that toothbrushing with herbal dentifrices reduces accumulation of supragingival plaque and resolves gingival inflammation, resulting in a number of these herbal agents having been incorporated into commercially available dentifrices and mouth rinses.

Similarly, Bromelain, a proteolytic enzyme derived from Pineapple (*Ananas comosus*) and Papain, a sulfhydryl protease derived from Papaya (*Carica papaya*) have been incorporated into dentifrices. The combination of papain and bromelain removes plaque by hydrolyzing the peptide bonds of the salivary pellicle and prevents plaque from adhering to the tooth surface.[5] The plaque inhibitory effect of papain and bromelain on the breakdown of the extracellular matrix of plaque potentially makes it effective in established biofilms.

Meswak (*S. persica*) and Neem (*A. indica*) extracts have antiseptic effect, preventing plaque formation.[6] In

Meswak, a natural component benzyl isothiocyanate is present that acts as an inhibitor of bacterial growth and their acidic products.[7] Neem has tetracyclic triterpenoids, which have shown similar antiseptic properties.[8]

AIM

To clinically and microbiologically evaluate and compare the plaque inhibitory effect of commercially available herbal extract containing fluoridated dentifrice to that of commercially available fluoridated dentifrice in a healthy dental cohort.

OBJECTIVES

To compare the plaque inhibitory effect of a commercially available herbal extract containing fluoridated dentifrice to that of a fluoridated dentifrice in a healthy dental cohort following 3 weeks in a cross-over study design with an intervening washout period of one week by evaluating the parameters of:

- Plaque index (PI))[9]
- Gingival index (GI)[10]
- Microbial colony-forming units (CFUs)

MATERIALS AND METHODS

The present study was a double-blinded, prospective, cohort, crossover study with the examiner and statistician being blinded. Ethical clearance was obtained from the Institutional Ethical Committee. The study population comprised 25 healthy dental student volunteers (5 males, 20 females). After being explained about the purpose, products to be used in the study, possible side effects, and study design, informed consent was procured through signed document. Participants were recruited for the study once they met the inclusion criteria.

The inclusion criteria were:

- Subjects aged 18 to 25 years rendering informed consent.
- Good level of oral hygiene, a mean Pl score < 1
- Presence of minimum 20 natural scorable teeth excluding third molars

Exclusion criteria were:

- Subjects showing any signs of plaque-induced gingival or periodontal disease
- Subjects who have received any form of periodontal therapy, surgical or nonsurgical within past 6 months
- Subjects who have received antibiotic and/or anti-inflammatory therapy within the past 1 month
- Subjects wearing orthodontic or prosthetic appliances
- Subjects who give a present or past history of drug abuse
- Subjects with untreated grossly carious teeth
- History of known allergies to constituents found in the dentifrice assigned during the investigation
- Subjects with a habit of mouth breathing, parafunctional habits like bruxism, clenching, etc
- Smokers and tobacco chewers

The following interventions were compared:

Dentifrice A: Commercially available fluoridated dentifrice (Colgate Cibaca Toothpaste, Colgate Palmolive, India) containing 1000 ppm of fluoride

Dentifrice B: Commercially available fluoridated dentifrice containing Bromelain, Papain, Meswak, and Neem (Glodent, Group Pharmaceuticals Ltd., Mumbai, India) containing 1000 ppm of fluoride

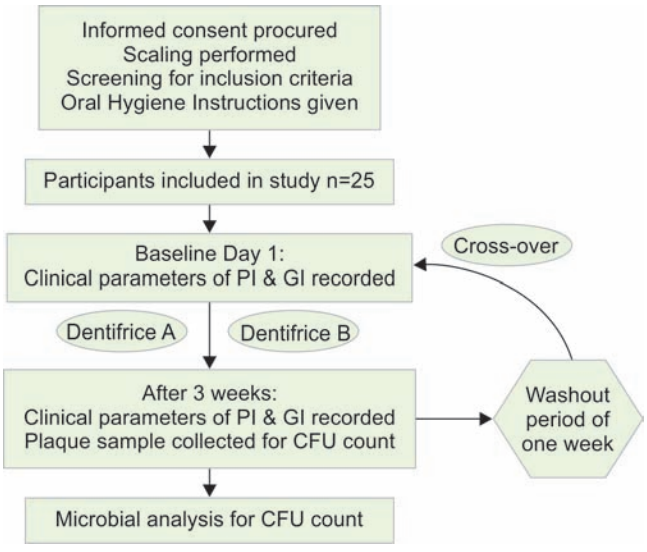
All the participants received ultrasonic scaling and polishing, and uniform oral hygiene instructions were given where subjects were asked to adopt modified bass brushing technique with a medium bristle toothbrush and prescribed the assigned dentifrice twice daily for 3 weeks. This regimen continued for all the participants throughout the duration of study. Clinical parameters of PI[9] and GI[10] were evaluated at baseline and at 3 weeks. At 3 weeks, a plaque sample from the lingual surface of the right mandibular first molar tooth surface of each participant was collected for microbial analysis through CFU count. To take the sample from the tooth surface, the quadrant was first isolated with cotton rolls and the lingual surface of the teeth was dried with a gentle stream of air. A cotton swab was stroked across the entire lingual tooth surface from mesial to distal while exerting pressure on the swab; an attempt was made to get as close as possible to the gingival margin without touching it. The collected swabs were stored in 1 mL of phosphate buffer saline in a refrigerator until analysis, which was done in the afternoon of the same day. For the analysis, the samples were vortex mixed for 30 seconds, and 100 µL of the 10^{-3} and 10^{-4} dilutions (phosphate-buffered saline) were used to inoculate Tryptone Soya Broth agar plates (Micromaster Laboratories Pvt. Ltd., Mumbai, India) using spread plate method. The plates were then incubated at 37°C for 24 hours. The results were expressed as CFUs/sample recorded with the help of a digital colony counting pen.

The participants were first assigned Dentifrice A for 3 weeks and all parameters were recorded. This was followed by an intervening washout period of 1 week where participants were asked to abstain from using any dentifrice while brushing, following which all participants were assigned Dentifrice B for a period of 3 weeks. Therefore, a total of two experimental phases in the crossover design were conducted with an intervening washout period of 1 week.

Participants were asked to maintain a diary to record any adverse events, such as discomfort while brushing, alteration in taste, and allergic reactions arising from the use of the products.

The detailed flow chart of the participants in the study is described in Flow Chart 1.

Flow Chart 1: Study events



Statistical Analysis

The data were tabulated and submitted to blinded statistician and analyzed using Statistical Package for the Social Sciences Software version 16. Based on data analysis, parametric tests for comparison were used. Paired t-test was used for intragroup comparison within Dentifrice A and Dentifrice B. Independent t-test was used for the intergroup comparison between Dentifrice A and Dentifrice B. A p-value less than 0.05 was treated as significant.

RESULTS

The data were tabulated and submitted to blinded statistician. The data for PI and GI were ordinal, whereas data for CFU count were numerical. Test of normality was applied using Kolmogorov–Smirnov test and Shapiro–Wilk test. The data were found to be noncomparable at baseline. Therefore, the difference in mean values at baseline and 3 weeks was used for intragroup and intergroup comparison.

On the intragroup comparison between baseline and 3 weeks for both Dentifrice A and Dentifrice B, the following observations were made. Both Dentifrice A and Dentifrice B were efficient in reducing PI and GI scores ($p < 0.05$) (Table 1).

On intergroup comparison for PI and GI, difference in mean was compared. It was observed that mean reduction in PI for Dentifrice B is significantly more than that of Dentifrice A (p-value: 0.000021). There was no significance of difference between the mean reduction in GI of both Dentifrice A and Dentifrice B (p-value: 0.3040) (Table 2).

Intergroup comparison of CFU between Dentifrice A (Fig. 1) and Dentifrice B(Fig. 2) was obtained from plaque sample after 3 weeks. It was observed that viable CFUs for Dentifrice B are significantly less than that of Dentifrice A (p-value: 9.42E-08) (Table 3; Graph 1).

DISCUSSION

The present study was designed to clinically and microbiologically evaluate and compare the plaque inhibitory effect of commercially available herbal extract containing fluoridated dentifrice (Dentifrice B) to that of fluoridated dentifrice (Dentifrice A) in a healthy dental cohort. Assessment of plaque inhibitory effect was based on scores of PI and GI. Microbiologically, it was assessed based on viable CFUs per sample collected from the plaque sample.

In the present study, both the dentifrices are able to significantly reduce PI scores and GI scores in the study population. For Dentifrice A, PI scores reduced by 0.01404 ± 0.006913 ($p < 0.05$) and for Dentifrice B, PI scores reduced by 0.02664 ± 0.011438 ($p < 0.05$).

Table 1: Clinical parameters at baseline and 3 weeks

	PI				*GI*			
	Dentifrice A		*Dentifrice B*		*Dentifrice A*		*Dentifrice B*	
	Baseline	*3 weeks*	*Baseline*	*3 weeks*	*Baseline*	*3 weeks*	*Baseline*	*3 weeks*
Mean	0.35664	0.3426	0.37968	0.35304	0.28	0.26016	0.28384	0.26016
Variance	0.00017874	0.00011475	0.000141143	0.00014229	0.000142833	0.000152057	0.00011664	0.000152057
t Stat	10.15473506		11.64559331		18.1194141		6.708708831	
p-value	3.63882E-10 (<0.05)		2.31886E-11 (<0.05)		1.66267E-15 (<0.05)		6.12958E-7 (<0.05)	

E: Exponential

Table 2: Comparison of reduction of clinical parameters at 3 weeks

	Reduction in PI		*Reduction in GI*	
	Dentifrice A		*Dentifrice B*	
Mean	0.01404	0.02664	0.01984	0.02368
Standard error	0.001383	0.002288	0.001095	0.00353
Standard deviation	0.006913	0.011438	0.005475	0.017649
Sample variance	4.78E-05	0.000131	3E-05	0.000311
p-value	0.000021 (<0.05)		0.3040 (>0.05)	

Table 3: Comparison of CFUs at 3 weeks

	Colony-forming units	
	Dentifrice A	*Dentifrice B*
Mean	3104800	2988200
Standard error	10577.02	13085.87
Standard deviation	52885.1	65429.35
Sample variance	2.8E+09	4.28E+09
p-value	9.42E-08 (<0.05)	

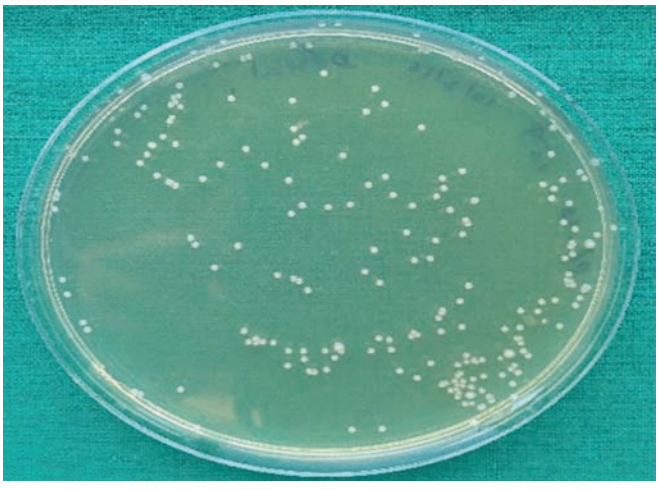


Fig 1: Colony-forming units obtained from plaque sample from participant using Dentifrice A at 3 weeks

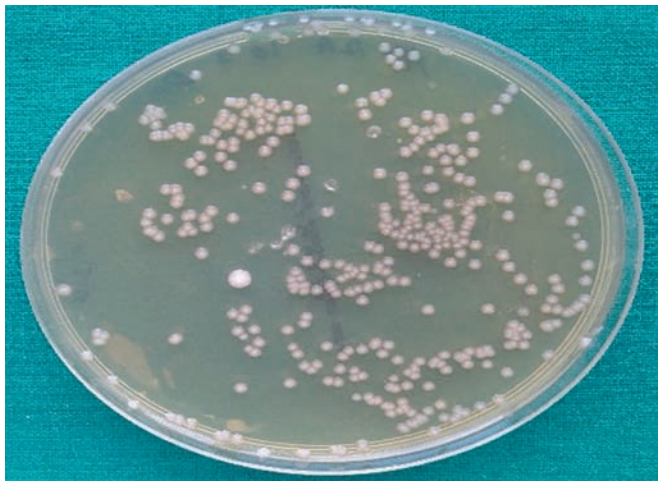
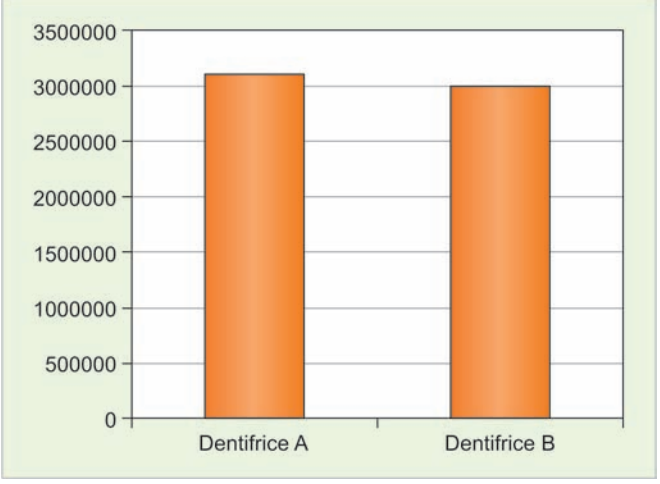


Fig 2: Colony-forming units obtained from plaque sample from participant using Dentifrice B at 3 weeks



Graph 1: Comparison of CFUs between Dentifrice A and Dentifrice B at 3 weeks

Both the dentifrices contained 1,000 ppm of fluoride. Fluoride is known to have antisurfactant effects, thereby preventing plaque adhesion.[11] This is in agreement with the studies of Mengel et al[12] who showed a reduction in plaque accumulation after use of fluoridated dentifrice.

In following the crossover design keeping factors of the tooth-brushing method, brushing duration, toothbrush type, and participant dexterity uniform, the greater difference in reduction of PI scores in Dentifrice A and Dentifrice B could be attributed to the effect of herbal extracts incorporated within the dentifrice. On intergroup comparison of the parameters, there was a statistically significant reduction in PI ($p = 0.000021$) score in favor of the herbal extract containing fluoridated dentifrice (Dentifrice B) as compared with the fluoridated dentifrice (Dentifrice A). This indicates additive/synergistic plaque inhibitory effect of the herbal extract.

This is in agreement with observations of Chakravarthy and Acharya,[5] who evaluated the efficacy of a novel dentifrice containing papain and bromelain extracts for antiplaque effect on established biofilm. It was inferred that bromelain- and papain-containing dentifrice showed significant pellicle removal, which could be attributed to the role of proteolytic enzymes as they remove the extracellular matrix. These results are also in agreement with those of Tatikonda et al[13] who found a significant reduction of plaque after using herbal extracts containing dentifrice.

However, a systematic review by Moran et al[14] concluded that herbal dentifrices had shown less plaque inhibitory effect than conventional fluoridated dentifrices.

The GI scores for Dentifrice A reduced by 0.01984 ± 0.005475 ($p < 0.05$) and for Dentifrice B reduced by 0.02368 ± 0.017649 ($p < 0.05$). The GI changed significantly for both the dentifrices (Table 1). However, there was no significant difference between the mean reduction in GI of Dentifrice A as compared with Dentifrice B ($p = 0.3040$) (Table 2).

However, Azaripour et al[15] compared the efficacy of a Miswak extract containing toothpaste with that of a herbal and a conventional fluoridated dentifrice, where a considerable reduction in the degree of gingival inflammation was seen in all dentifrices.

The CFU count on 24 hours incubation revealed significantly reduced levels of viable CFUs in herbal extract containing fluoridated dentifrice (Dentifrice B) as compared with fluoridated dentifrice (Dentifrice A) ($p = 9.42E-08$) (Table 3). This can be attributed to the antiseptic effect of the herbal extract of Meswak and neem.

This is in agreement with the studies of El-Desoukey,[16] who investigated the efficiency of antimicrobial effect of Meswak-containing dentifrice. It was demonstrated that Meswak is an efficient antimicrobial agent. Patil et al[17] compared the antimicrobial effect of dentifrice containing neem with that of fluoridated dentifrice, wherein it was found that dentifrice containing Neem as well as fluoridated dentifrice are equally efficacious against bacteria.

However, Bhati et al[18] evaluated the antimicrobial efficacy of fluoridated and herbal dentifrices containing Meswak where there was no significant difference observed in the antimicrobial property of the test dentifrices.

CONCLUSION

As per the current study settings, it was observed that herbal extracts in dentifrices have an additive effect with fluoride in plaque inhibition. This could be attributed to bromelain and papain-induced enzymatic breakdown that acts on the extracellular matrix, thereby preventing maturation of the plaque biofilm and the antiseptic effects of Meswak and Neem. The antiseptic effect of Meswak and neem was further displayed by a reduction in CFU in the herbal extract containing dentifrice as compared with the fluoridated dentifrice.

LIMITATIONS

As the subjects were from the dental cohort, Hawthorne effect could possibly have contributed to good plaque control in both groups; however, the crossover study design was aimed at minimizing this effect.

Anaerobic culture of plaque sample was not performed.

CLINICAL SIGNIFICANCE

Herbal extracts in dentifrices have an additive effect with fluoride in plaque inhibition.

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