



## **Effect of Two Different Mouth Rinses on *S. mutans* Counts in Subjects Undergoing Orthodontic Treatment – A Pilot Study**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author SA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RKJ and ASSG managed the analyses of the study. Author ASSG managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

The study is thus aimed to assess and compare the efficacy of Herbostra oil pulling mouthwash with Chlorhexidine mouthwash in reducing plaque accumulation around orthodontic brackets. A total of 20 patients were considered in this study randomly assigned into Group I (experimental group - Herbostra oil pulling mouthwash) and Group II (reference group-0.2% Chlorhexidine mouthwash). The plaque index scores were recorded in each individual at baseline (pre) and after 3 weeks (post). Dental plaque samples were collected around the orthodontic brackets at the cervical region of maxillary upper molars and lower incisors by cotton swabbing method and evaluated for the presence of microflora. Paired sample t-test for *Streptococcus mutans* count showed that statistically significant difference only within the group II (p=0.000) (Chlorhexidine group) and there was no significant difference within the group I (p=0.103) (Herbostra group). Paired sample t-test for plaque index score shows statistically significant difference within the

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groups (0.000).Independent t test showed statistically significant difference in the levels of *Streptococcus mutans* count after 3 weeks between the two groups ( $p=0.000$ ) with the mean values of  $(2.230\pm 0.5056)$ ,  $(1.080\pm 0.3458)$  in group I and group II respectively. From this study we concluded that, even though there was a reduction in plaque scores and *S. mutans* count with Herbostra oil pulling mouthwash but it was not as effective as Chlorhexidine mouth rinse.

**Keywords:** *Streptococcus mutans*; dental plaque; orthodontic appliances; Chlorhexidine; oil pulling.

## 1. INTRODUCTION

Plaque and calculus accumulation around orthodontic brackets is very commonly noted during routine orthodontic visits. Dental plaque consists of a variety of microbes and among them *Streptococcus mutans* is considered most virulent and is associated with white spot lesions, dental caries and gingival inflammation [1]. Maintaining oral hygiene is a difficult task for orthodontic patients due to the presence of attachments such as bands, wires, and ligatures [2]. Orthodontic appliances act as mechanical hindrances for plaque removal by the action of brushing, mastication, and salivary flow [3]. If patients are not positively motivated regarding oral hygiene then it will lead to the development of hyperplastic gingivitis within 1 to 2 months [4] even attachment loss has been noted after the removal of appliances [5,6] which can be reversed with proper preventive measures [7].

Chemical agents could be used in addition to mechanical agents such as brushing and flossing during the active phase of orthodontic treatment to reduce the bacterial plaque accumulation, gingivitis and periodontitis [8]. Antimicrobial mouth rinses like Chlorhexidine have been introduced as an effective method for reducing dental plaque accumulation [9]. Chlorhexidine mouthwash can be considered as the most popular mechanical intervention during management of gingivitis and proved to be very effective in the reduction of plaque accumulation [10] and regarded as 'Gold standard' for plaque control [11]. The superiority of this agent as opposed to other chemical agents is substantivity that prolongs its antibacterial action used for plaque control [12]. However, long-term use of CHX products is associated with local side effects such as altered taste, tooth staining, increased formation of supragingival calculus, occasional irritation and desquamation of mucous membranes [13].

To overcome these side effects certain other mouth rinses have been introduced. Oil pulling or oil swishing is an ancient natural healing practice

originated in India. Few studies showed efficacy of oil pulling as an antimicrobial agent using edible oils such as sesame oil, coconut oil, and sunflower oil that have shown to reduce the severity of plaque-induced gingivitis [14–16]. Sesame oil is known for its beneficiary effects and is found to have the antibacterial activity against *S. mutans* and many other bacteria [17]. A commercially available sesame oil based oil-pulling mouthwash by Herbostra have been recently introduced for oral hygiene maintenance and it claims reduction of plaque accumulation. Our extensive research expertise ranged from epidemiological studies to randomized clinical trials that have been published in reputed journals [18–27]. This knowledge was instrumental for us to study the effect of two different mouth rinses on *Streptococcus mutans* in subjects undergoing Orthodontic treatment.

The aim of this study is to assess and compare the efficacy of Herbostra oil pulling mouthwash with Chlorhexidine mouthwash in reducing plaque accumulation around orthodontic brackets.

## 2. MATERIALS AND METHODS

This study was carried out at the Department of Orthodontics, Saveetha Dental College by two investigators. The study was approved by the scientific review board of Saveetha dental college and hospitals.

Subjects of both genders in the age range of 18-25 years undergoing orthodontic treatment with pre-adjusted edgewise appliance and having equal baseline plaque index scores were randomly included in the study. Subjects with systemic diseases like diabetes mellitus, subjects who smoke or chew tobacco were excluded. Subjects with pre existing gingival and periodontal problems, under antibiotics and oral mouth rinses during the last 3-month period were also excluded.

Subjects were randomly assigned into two groups, Group I experimental group - Herbostra

oil pulling mouthwash) and Group II (reference group-0.2% Chlorhexidine mouthwash) and 10 subjects were included in each group. A total of 20 subjects with almost equal baseline mean scores were chosen for the study and the mouth rinses wrapped by a white sticker were given to them. Both the investigators and the study subjects were blinded.

All participants were instructed to use the mouth rinse according to its manufacturer's instructions for 3 weeks. In group I the study subjects were asked to take 5 ml of Herbostra oil pulling mouthwash and swish it around the mouth, teeth, gums and tongue for 10 min then spit it out and this was done after meals twice a day for three weeks. In group II the study subjects were asked to rinse with 5 mL of 0.2% Chlorhexidine mouthwash twice daily after meals for 3 weeks.

The plaque index scores were recorded in each individual at baseline (pre) and after 3 weeks (post).

## 2.1 Sample Collection

The following method was used in sample collection for microbial analysis. Quadrants were isolated with cotton rolls to avoid saliva contamination before collecting the sample. Plaque samples were collected aseptically with the help of sterile cotton swab moistened with sterile saline from cervical region around the orthodontic attachments of maxillary upper molars and lower incisors. Only one swab was used for both regions and one sample was collected from each patient. The swab was transferred aseptically and immediately into the sterile tube containing 2 ml of BHI (Brain Heart Infusion) broth medium and it was delivered to the microbiology laboratory (Fig. 2).

## 2.2 Estimation of Microbial Counts

Swab was inoculated into the tube containing 2ml of BHI broth for bacterial isolation and identification; it was incubated at 37°C for 2 hrs. After 2 hours, 10 µl of the broth was inoculated onto fresh Mutans-Sanguis agar for *Streptococcus mutans* isolation. The culture plates were then incubated at 37°C for 24 hours for *Streptococcus mutans*. After bacterial growth, colony morphologies were evaluated, counted and measured in colony forming units (cfu/ml) (Fig. 3).

## 2.3 Statistical Analysis

The data obtained during the course of the study was systematically entered in the Microsoft Excel sheet. Data analysis was performed using SPSS Software (IBM Corp, Version 13). Data was normally distributed, so parametric tests have been employed. Independent paired "t" test was employed to compare the mean and standard deviation of *Streptococcus mutans* count between the groups. Paired t test was done to compare the mean and standard deviation of *streptococcus mutans* count within the group.

## 3. RESULTS AND DISCUSSION

Table 1 & Fig. 1, gives the results of Paired sample t-test for *Streptococcus mutans* count pre and post in group I and group II shows statistically significant difference only within the group II (0.000) (Chlorhexidine group) and there was no significant difference within the group I (0.103) (Herbostra group).

Table 2 gives the results of Independent t test for comparison of *Streptococcus mutans* count after 3 weeks between group I (Herbostra group) and group II (Chlorhexidine group) and it showed statistically significant difference in the levels of *Streptococcus mutans* between the two groups (0.000) with the mean values of (2.230±0.5056), (1.080±0.3458) in group I and group II respectively.

Table 3 gives the results of Paired sample t-test for plaque index score pre and post in group I and group II shows statistically significant difference within the groups (0.000).

Orthodontic treatment is associated with poor oral hygiene and significant increase in the number of *streptococcus mutans* and Lactobacilli [28,29]. Excess composite around the bracket base and presence of a distinct gap at the composite-enamel interface is a site for plaque accumulation making it necessary to sample the plaque from the tooth surface [30]. Antimicrobial mouth rinses have been introduced as an effective method for reducing dental plaque accumulation in orthodontic patients [9], and among them Chlorhexidine is considered gold standard. However, long-term use of CHX products is associated with local side effects such as altered taste, tooth staining, increased formation of supragingival calculus, occasional irritation and desquamation of mucous membranes [13].

**Table 1. Paired sample t-test - comparison of mean and standard deviation of *Streptococcus mutans* count within the groups**

		N	Mean	Standard Deviation	Significant difference (p value)
Group I	Oil pre	10	2.62	0.3765	0.103
	Oil post	10	2.23	0.5056	
Group II	CHX pre	10	2.74	0.3098	0.000*
	CHX post	10	1.08	0.3458	

*p* value –probability value

\* Statistically significant at  $p < 0.05$  (2-tailed)

**Table 2. Independent sample t-test -comparison of mean and standard deviation of *Streptococcus mutans* count between the groups**

	Mouthwash	N	Mean	Standard Deviation	Significant difference (p value)
Post	Oil	10	2.230	0.5056	0.000*
	CHX	10	1.080	0.3458	

*p* value –probability value

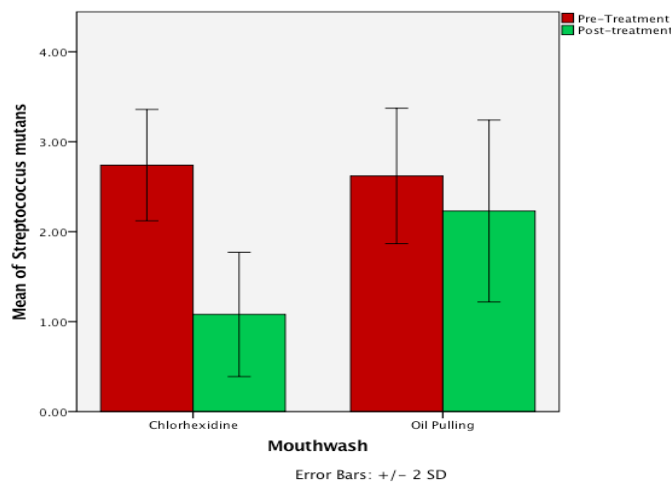
\* Statistically significant at  $p < 0.05$  (2-tailed)

**Table 3. Comparison of mean and standard deviation of plaque index within the groups**

		N	Mean	Standard Deviation	Significant difference (p value)
Group I	Oil pre	10	1.46	0.2119	0.000*
	Oil post	10	0.75	0.3440	
Group II	CHX pre	10	1.46	0.2221	0.000*
	CHX post	10	0.57	0.3302	

*p* value –probability value

\* Statistically significant at  $p < 0.05$  (2-tailed)

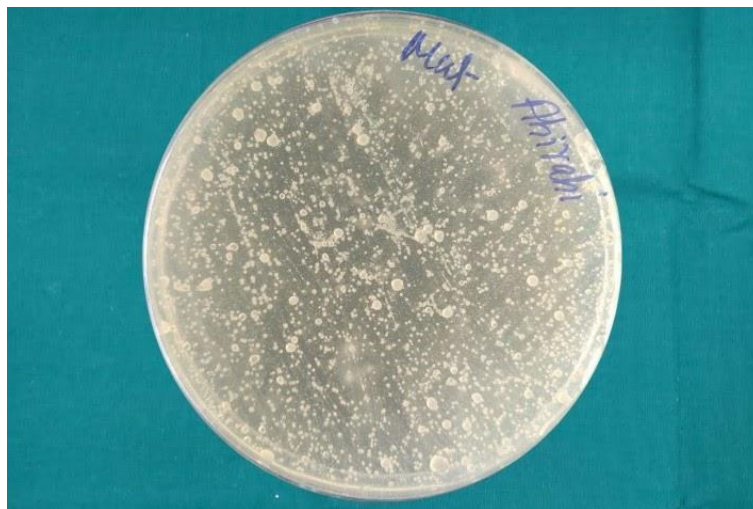


**Fig. 1. Bar chart representing the comparison of means of pre-treatment and post-treatment *Streptococcus mutans* in both Chlorhexidine and Herbostra oil pulling mouthwash**

The X-axis represents the Chlorhexidine and Herbostra oil pulling mouthwash. The Y-axis represents the mean of *Streptococcus mutans* and their respective standard deviations. Paired t-test was performed and it was found to be statistically significant with  $p$  value -0.000. It was inferred that there was a decrease of  $2.74 \pm 1.08$  mean values in streptococcus mutans count in group II (Chlorhexidine group) whereas there was a slight decrease of  $2.62 \pm 2.23$  mean values in streptococcus mutans count in group I (Herbostra group) and this was found to be statistically not-significant ( $p > 0.05$ )



**Fig. 2. Collection of specimens in a transport vial with broth from different sites on the oral cavity from the orthodontic patients**



**Fig. 3. Estimation of colony counts from MS agar to determine the colony forming units at different sites**

The present study aimed at comparing the antimicrobial and anti plaque effect of a novel commercially available oil pulling mouth rinse with Chlorhexidine mouth rinse. The mouth rinse tested in this study is sesame oil based and since sesame oil is a proven antimicrobial we had attempted to check its efficacy in reducing salivary *S. mutans* count and plaque scores and compare it with Chlorhexidine. The results of this study report that oil pulling mouth rinse was as effective as Chlorhexidine in reducing plaque scores but not effective in reducing *S. mutans* count. In both groups the *S. mutans* score had reduced after 3 weeks but only in the Chlorhexidine group there was a statistically

significant reduction in *S. mutans* count noted at the end of 3 weeks time, but this was not so in the oil pulling group. Nevertheless Herbostra oil pulling mouthwash can be considered for using in orthodontic patients as the treatment duration is longer and Chlorhexidine cannot be used for a duration of more than 2 weeks since it is associated with many problems like taste, staining of teeth and opportunistic Fungal infections.

In the present study plaque samples were collected using cotton swabs since it is validated and a low-cost practical method [31]. Since plaque accumulation is more in the lower anterior

region and upper molar region as compared to other regions of the oral cavity the samples were collected from these sites only [32].

Oil pulling is a traditional Indian folk remedy and it is an ayurvedic practice that involves swishing of oil in the mouth for oral and systemic health benefits [33]. Reduction of bacterial count by about 20% and moderate antimicrobial activity against *Streptococcus mutans* and *Lactobacillus* on 40 days of oil pulling using sesame oil has been reported previously [34]. An in-vitro study showed that sesame oil possesses antibacterial activity against *S. mutans* on oral biofilm model [16]. The saponification and emulsification process during oil pulling is responsible for cleansing action of sesame oil [35]. It does not cause staining, altered taste and allergy unlike Chlorhexidine. However the duration of the procedure is longer compared to Chlorhexidine [36]. Sesame oil showed comparative effectiveness as Chlorhexidine against plaque induced gingivitis and also in reducing halitosis [36,37]. Hence in this present study, Herbostra oil pulling mouthwash formulated with sesame oil and 25 potent herbs was tested for its effectiveness in orthodontic patients.

The limitations of this study include a smaller sample size, shorter duration, only *S. mutans* count was evaluated, reliability on patients for using the mouth rinse regularly, individual differences in using the mouth rinse.

Further studies with larger sample size in many centers can be more authentic and applied for the general population. Studies on comparison with fluoride mouth rinses are needed in the near future.

#### 4. CONCLUSION

Within the limitations of this study we can conclude that even though there was a reduction in plaque scores and *S. mutans* count with Herbostra oil pulling mouthwash but it was not as effective as Chlorhexidine mouth rinse.

#### CONSENT

Informed consent was obtained from participants after explaining the study.

#### ETHICAL APPROVAL

As per university standard guideline, ethical approval have been collected and preserved by the authors

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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