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ORIGINAL ARTICLE

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The effect of herbal, essential oil and chlorhexidine mouthrinse on *de novo* plaque formation

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Abstract: *Background:* Brushing and flossing are the most widely accepted procedures, the 'gold standard', for controlling bacterial plaque, but these mechanical methods have limitations. Based on results derived from several clinical trials, essential oil (EO) mouthrinse (Listerine®) and a chlorhexidine mouthrinse have been accepted by ADA to be used as an adjunct to routine mechanical oral hygiene measures however, both of them are associated with side effects, therefore, the present study was undertaken to evaluate the antiplaque efficacy of a new herbal formulation as compared to an EO and chlorhexidine rinse. *Materials and method:* The study was a single blind parallel randomized controlled trial involving four groups. 48 volunteers refrained from all oral hygiene measures for 4 days, but rinsed instead twice daily with 10 ml of a herbal (HM), EO, chlorhexidine (CHX) or a placebo (PL) solution. Plaque index and plaque area (PA) was assessed on Day 4. *Results:* The HM and EO showed a significant inhibition of plaque regrowth compared to PL ($P < 0.001$), but the lowest values of PI and PA were obtained with CHX. Statistically significant difference in plaque parameters was observed when CHX was compared to HM and EO, and HM to EO rinse. *Conclusion:* The new herbal mouthrinse had a promising plaque inhibitory potential but it not as efficacious as chlorhexidine in preventing plaque regrowth.

Key words: plaque; mouthrinse; herbal; essential oil; chlorhexidine

Introduction

Dental plaque is an adherent bacterial biofilm that forms on hard and soft tissues intra-orally (1). Its inadequate control is one of the primary causative factors in the development of gingivitis and periodontal disease progression. While mechanical methods of plaque removal are considered the standard for individually applied oral disease preventive practices, the high prevalence of gingival disease has prompted research into and development of adjunctive methods for controlling oral biofilms. In 2002, data presented at the International Association for Dental Research (IADR) meeting supported the benefit of oral rinsing with chemotherapeutics as an adjunct for controlling plaque and maintaining gingival health (2).

A large number of commercial plaque control agents are available, but none are without shortcomings. Chlorhexidine digluconate (CHX), currently recognized as a gold standard, is still examined intensely, either combined with other ingredients or as a positive control (3–5). However, its duration of use is limited to just a few weeks because of undesirable side effects such as taste disturbances, tooth discolouration and, less

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commonly, desquamation of oral mucosa (6). Besides chlorhexidine rinses, only essential oil (EO) rinses have been extensively evaluated and subsequently shown to be of value as an adjunct to mechanical oral hygiene procedures. Literature is replete with evidence about its detrimental oral effects such as epithelial detachment, keratosis, mucosal ulceration, petechiae and oral pain which can be attributed to high alcohol content, thus restricting its prolonged use (7, 8). Hence, the quest for a long-term, ideal and safe antiplaque and antigingivitis agent continues.

In this context, synthetic antimicrobials have been analysed, but the increasing problems of resistance have encouraged the search for alternative agents based on herbal extracts.

HiOra is a complete herbal mouthrinse (HM) that contains bibhitaki, nagavalli, pilu, peppermint satva, yavani satva, gandhapura taila and ela. Research has shown that pilu (*Salvadora persica*), one of the primary constituent, possess antiplaque activity that is comparable to that of chlorhexidine (9). Nagavalli (*Piper betle*) has also been proven to have plaque inhibitory activity in in-vitro studies (10, 11). Other constituents (bhibhitika, peppermint satva, gandhapura taila) have encouraging antimicrobial activity that may be helpful in providing better oral care (12, 13).

On the basis of these researches, the aim of this study was twofold: firstly, to evaluate the plaque inhibitory efficacy of a herbal formulation as compared to a chlorhexidine (positive control) and a placebo (negative control) mouthrinse; secondly, to include an established and commercial available product (Listerine®) for a further useful comparison.

Materials and method

Study population

Forty-eight volunteers (20 male and 28 female students; age range 21–26 years, of the Department of Periodontology, Faculty of Dental Sciences, CSMMU) participated in the study. The volunteers had a minimum of 25 scorable teeth and documented high standard of oral hygiene and gingival health. The presence of grossly carious teeth, more than one full coverage restoration, fixed or removable orthodontic appliances or partial dentures, poor oral hygiene [papilla bleeding index (PBI) of more than 30%], pocket >5 mm or attachment loss >2 mm, known intolerance or allergy to mouthrinses and use of antibiotics or medications in last 3 months that might interfere with plaque formation were the exclusion criteria.

All eligible volunteers were given oral and written information about the products and the purpose of the study and were asked to sign an informed consent. The study was conducted in accordance with ethical principles originating in the Declaration of Helinski and consistent with good clinical practice.

Study design

This clinical study used an examiner/observer blind, randomized, four group and parallel design in a 4-day plaque regrowth model. The subjects were randomly divided into four groups (12 each), through computer-generated random numbers, and one of the four mouthrinses was assigned to each group. The allocation of active or control solutions was carried out by a person not directly involved in the research project.

At baseline (Day 0), after oral soft and hard tissue examination, plaque was disclosed using disclosing solution (MIRA-2-TON; Hager & Werken, Duisburg, Germany), and all the participants received a thorough scaling and polishing to remove all plaque, stain and calculus, using ultrasonic scalers and hand instruments. Special attention was paid at interproximal areas where dental floss was used. To ensure that all deposits had been removed a second disclosing episode was carried out after which remaining plaque was removed. The volunteers then rinsed for 1 min with 10 ml of their allocated rinse. All normal oral hygiene procedures were then suspended for the next 4 days, and subjects were instructed to rinse two times a day, after breakfast and in the evening, for 1 min with 10 ml of their assigned rinse.

On Day 4, the subjects were recalled and were asked to bring back the bottles so that their compliance can be assessed by measuring the residual mouthwash in them. All the subjects received a re-examination of their oral soft and hard tissue and were scored and photographed for assessment of plaque. After assessment, participants received another rubber cup polishing to remove all plaque and tooth stain, if present.

Test solutions

Herbal mouthrinse: A polyherbal composition with each gram of mouthwash containing (i) Extracts: Bhibitaka (*Terminalia bellerica*, 10 mg), Nagavali (*P. betle*, 10.0 mg) Pilu (*S. persica*, 5.0 mg); (ii) Powders: Peppermint Satva (*Mentha* spp., 1.6 mg), Yavani satva (*Trachyspermum ammi*, 0.4 mg) and (iii) Oils: Gandhapura taila (*Gaultheria fragrantissima*, 1.2 mg), Ela (*Elettaria cardamomum*, 0.2 mg). (HiOra; Himalaya Herbal Healthcare, Bangalore, India)

Essential oil mouthrinse: Constitutes of EOs like menthol 0.042%, thymol 0.064%, eucalyptol 0.092%, methyl salicylate 0.060%. (Cool mint Listerine® mouthwash, Lamurkka Pathumthani, Thailand)

Chlorhexidine mouthrinse (CHX) (functional positive control): A commercially available non-alcoholic 0.2% chlorhexidine mouthwash (Chlohex; Dr Reddy's Lab Ltd., India).

Placebo mouthrinse (PM) (negative control): Distilled water was coloured to resemble mouthwash.

All the test solutions were predispensed in the identical bottles for total subject masking.

Clinical evaluation

To test the influence of the test solutions on plaque regrowth, parameters recorded were plaque index (PI) according to Turesky *et al.* (14) modification of Quigley Hein PI (15) and plaque area (PA).

For PI, the scores were taken at six surfaces per tooth: mesio-, mid- and disto – buccal, and mesio-, mid- and disto – lingual after staining plaque with disclosing solution. All teeth except 3rd molars were examined, and all scorings were carried out by the same investigator who was unaware of the allocation of the mouthrinse to participants.

Plaque area was evaluated after 4 days by calculating the percentage of plaque-covered area to total tooth area. After staining with disclosing solution, digital standardized photography and computer-based calculation were performed. For this study, only the upper right and left lateral incisors were selected. The stained buccal surface was highlighted on the digital photograph using the Adobe photoshop CS5 extended (12.1 × 3.2 version), and then, the number of pixels within the area was calculated. The relation between the plaque-covered labial area (number of pixels) and the total vestibular labial tooth surface (number of pixels) gave the percentage of existing plaque (16).

The PBI by Saxer and Muhlemann (17) was assessed at the buccal sites of the gingiva of all teeth on day 0 and day 4 as a control parameter to evaluate the gingival health of the subjects during the whole test period.

In addition, any adverse effects during the use of mouthrinses were recorded on a question sheet on a scale from absent, mild, moderate or severely present.

Statistical evaluation

After the test period is completed and the mouthrinse order decoded, the evaluation was performed using the computer program Statistical Package for Social Sciences (SPSS) Version 15.0 (SPSS Inc., Chicago, IL, USA) and Lead Tools© 1991–2000 (LEAD Technologies Inc., Chicago, IL, USA). The mean values of clinical parameters (PI and PA) were calculated for each rinse solution. First analysis of variance (ANOVA) was performed to determine the differences among products tested. In the presence of significant differences, pairwise comparisons were made via Tukey HSD. The Tukey HSD was used as the *post hoc* test to control the possibility of alpha-error owing to smaller sample size. The confidence level of the study was kept at 95%; hence, a '*P*' value < 0.05 indicated statistically significant differences.

Results

Compliance

There was no drop out and all the 48 subjects completed the trial. Though the rinsing, with the exception of the first one, was not supervised, the amounts of mouthrinses used indicated good compliances with the instructions. With the aid of questionnaire, it was observed that all rinsing solutions were accepted by the participants. No adverse events or side effects were reported or observed except occasional staining that was restricted to the use of CHX rinse.

Table 1. **Mean values of plaque parameters (PI and PA) after 4 days of *de novo* plaque formation**

	PI			PA (%)		
Group	Mean ± SD	Min.	Max.	Mean ± SD	Min.	Max.
HM	2.94 ± 0.12	2.78	3.14	41.62 ± 2.79	37.50	45.81
EO	3.21 ± 0.10	3.04	3.33	45.93 ± 2.37	42.38	49.83
CHX	2.69 ± 0.11	2.52	2.86	38.21 ± 2.27	34.61	41.67
PL	3.77 ± 0.05	3.70	3.86	53.81 ± 2.95	49.38	58.21
	$F = 249.098$; $P < 0.001$			$F = 79.681$; $P < 0.001$		

HM, herbal mouthrinse; EO, essential oil mouthrinse; CHX, chlorhexidine mouthrinse; PL, placebo mouthrinse; PA, plaque area; PI, plaque index.

Plaque regrowth inhibition

The mean PI and PA for each group after 4 days of *de novo* plaque formation is shown in Table 1. The positive control (CHX solution) attained the lowest values for plaque parameters (PI 2.69; PA 38.14%) while the highest were achieved by negative control (PI 3.77; PA 53.81%). The mean PI and % PA for HM and EO rinse were 2.94, 41.62, 3.21 and 45.93, respectively.

Differences between the individual rinse solutions and the placebo solution, determined via Tukey HSD test, are demonstrated in Table 2. Compared to placebo, CHX, HM and EO rinse resulted in significantly less plaque regrowth. Statistically significant difference in plaque parameters was observed when CHX was compared to HM and EO, and HM to EO rinse.

Discussion

The incorporation of broad spectrum antimicrobial mouthrinses as adjuncts to patient's daily oral hygiene regimens has assumed greater importance with the recognition that most individuals are unable to consistently maintain adequate levels

Table 2. **Differences between the active and PL in PI and PA on day 4**

	Dependant variables			
	PI (SE −0.04156)		PA (SE −1.06672)	
Group	Mean difference	*P*-value	Mean difference	*P*-value
HM versus EO	−0.26917	<0.001	−4.31083	0.001
CHX	0.24417	<0.001	3.41167	0.013
PL	0.83583	<0.001	−12.19083	<0.001
EO versus CHX PL	0.51333	<0.001	7.72250	<0.001
PL	−0.56667	<0.001	7.88000	<0.001
CHX versus PL	−1.08000	<0.001	−15.60250	<0.001

HM, herbal mouthrinse; EO, essential oil mouthrinse; CHX, chlorhexidine mouthrinse; PL, placebo mouthrinse; PI, plaque index; PA, plaque area.
The mean difference is significant at the 0.05 level.

of plaque control using mechanical methods alone (18). As a result, various synthetic and herbal antimicrobial rinses have been developed and marketed, amongst which, 0.2% chlorhexidine, EO (Listerine®) and a herbal (HiOra) mouthrinse, was included in the comparative plaque regrowth study reported herein.

This study was undertaken to analyse the plaque inhibitory potential of HM and to rank this formulation against a negative (placebo) and positive (CHX) as well as a benchmark control. Listerine® (EO rinse) was chosen as a benchmark control, as its efficacy in controlling plaque has been documented by numerous clinical trials, both short- and long-term (19–22).

The 4-day plaque regrowth model was chosen for the present endeavour as it has been employed in numerous investigations and can be described as an established method for assessing the plaque inhibitory activity of formulations *per se* and determines the relative efficacy of different formulations (3, 20, 21, 23). The study design measures the plaque regrowth under the influence of test solution from a zero plaque baseline and avoids the confounding influences of tooth-brushing, which is highly variable between individuals. If no plaque inhibition can be shown in this type of study, no further effect of the rinsing solution can be expected in studies where oral hygiene is performed (23).

In this study, the HM showed plaque inhibition which lay between the negative and positive control (Table 1). A statistically significant suppression of *de novo* plaque formation was seen with herbal formulation as compared to placebo solution ($P < 0.001$) and EO rinse ($P < 0.001$) (Table 2). The plaque preventing potential of HM, demonstrated in the present investigation, can be attributed to its constituents like *S. persica*, *P. betle*, *T. bellerica*, *E. cardamomum* and others.

Salvadora persica, toothbrush tree, locally called miswak has been proven as an antiplaque agent by numerous studies (9, 24). Its antiplaque activity might be due to its antimicrobial activity against early and late plaque formers. Sofrata *et al.* (25) reported antibacterial effect of *S. persica* against oral pathogens such as *Streptococcus mutans*, *Lactobacillus acidophilus*, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Haemophilus influenza*. These findings are also supported by the studies of Darout *et al.*, Almas and Al-Zaidi, and Al-Bayati and Sulaiman (26–28). However, when antimicrobial effect of *S. persica* and CHX against *S. mutans* was compared, chlorhexidine was found to be more potent in reducing the number of *S. mutans* colonies (29).

Another constituent of HiOra herbal mouthwash, *P. betle*, has been documented to reduce significantly the cell-surface hydrophobicity of three early plaque settlers such as *Streptococcus mitis*, *Streptococcus sanguis* and *Actinomyces* sp. (11, 30). This cell-surface hydrophobicity is well established as a factor involved in the adherence of bacteria to the host tissues (31). Furthermore, *E. cardamomum* has been reported to significantly inhibit the growth of oral microflora in vitro studies (32, 33). Besides the above-mentioned ingredients, *T. bellerica*, *Mentha* Spp. *G. fragrantissima* has also proven to possess antimicrobial activity that might contribute to the antiplaque activity exhibited by the HM (12, 13, 34).

In this study, though the herbal formulation achieved a significant reduction in plaque regrowth when compared to negative and benchmark control, it was not as efficient as the gold standard (CHX) antiplaque mouthrinse. This difference in reduction observed between the HM and CHX reached the statistical significance. Because of new formulation of the mouthwash used, direct comparisons of the results obtained are not possible. Gazi *et al.* (24) compared antiplaque effects of pilu (*S. persica*) and 0.2% chlorhexidine rinse using a different study design and found CHX to be superior over *S. persica* slurry in inhibiting plaque. Moreover, Rahmani *et al.* in 2005 (9) also assessed the effects of *S. persica* and CHX on plaque formation; however, they noted a comparable plaque inhibition by both the solutions.

The response of participants to the herbal product, as evaluated by questionnaire, was good. In addition, no side effects had been noticed at the end of the study, which might add to its clinical usage as an adjunct to mechanical oral hygiene measures.

On the basis of the results obtained, it can be stated that this new HM had a promising plaque inhibitory potential, and however, studies of longer duration in which the antigingivitis activity of product in question is also assessed along with its antiplaque property and where safety and microbiological parameters will be evaluated are essential to establish the true effectiveness of this mouthrinse and its position among the other rinses that are used adjunctively to mechanical oral hygiene procedures.

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