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ORIGINAL RESEARCH

Evaluation of the Antimicrobial and Anti-inflammatory Efficacy of Two Commercially Available Mouthwashes

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ABSTRACT

Introduction: Various chemical agents are being used as an adjunct to mechanical therapy. Chlorhexidine (CHX), though considered as the gold standard, has certain side effects. The use of herbal products as an adjunctive therapy is thus gaining more popularity. The aim of this study was to evaluate the antimicrobial and anti-inflammatory efficacies of two commercially available mouthwashes.

Materials and methods: Antimicrobial activity of the mouthwashes was analyzed *in vitro* by evaluating their minimal inhibitory concentration (MIC) against four microorganisms. The anti-inflammatory efficacy was evaluated in 84 individuals, who were divided into four groups of 21 each based on their gingival index (GI) score. The subjects used the respective mouthwashes allotted to them for 2 weeks. Plaque index (PI), GI, and modified sulcus bleeding index (mSBI) of the participants were recorded at baseline and 14 days.

Results: The herbal mouthwash was effective against the tested organisms *in vitro*. The PI and GI scores reduced in all the four groups at the end of 14 days with the CHX group showing more reduction. The herbal mouthwash showed significantly better clinical outcomes when compared with other groups and results comparable to CHX.

Conclusion: The herbal mouthwash showed antimicrobial and anti-inflammatory effects comparable with that of 0.2% CHX, and may have a potential as an adjunct to scaling and root planing.

Keywords: Anti-inflammatory agents, Antimicrobial agents, Chlorhexidine, Gingival index, Gingivitis, Mouthwashes.

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INTRODUCTION

Gingivitis, the mildest form of periodontal disease, is highly prevalent and readily reversible by simple, effective oral hygiene and affects 50 to 90% of adults worldwide, depending on its precise definition.[1] It is an established fact that accumulation of debris leads to gingival inflammation, and bacteriological studies have indicated that the difference between the microbial floras of the healthy gingivae as compared with inflamed gingivae is mainly quantitative, although minor differences in the relative composition of the flora have been observed.[2] Though considered as a physiologic inflammatory response to plaque accumulation, gingivitis is the starting point for more serious and destructive periodontal diseases.[3,4] Hence, it is most important that periodontal disease be recognized and treated in the early stages, when the manifestations are most easily reversed and major damage to the periodontium has not yet occurred. Periodontal treatment, thus, focuses on the thorough removal of plaque, calculus, and plaque products.[5]

Mechanical removal of the plaque remains to be the mainstay of periodontal therapy, which can be done professionally or through homecare products. Chemical agents that constitute the chemical plaque control measures act as an adjunct to mechanical plaque control. Chlorhexidine is regarded as the gold standard against which other antiplaque and gingivitis agents are measured.[6] The CHX, though very effective, also has certain side effects like brown discoloration of the teeth, oral mucosal erosion, and bitter taste.[7] Hence, the quest for a long-term antiplaque and antigingivitis agent continues.

The World Health Organization guidelines define herbal medicines as finished labeled medicinal products containing an active ingredient, i.e., obtained from the aerial or underground parts of botanicals or other plant materials or their combination. There has been a resurgence of interest in the use of natural substances as effective antiplaque and antigingivitis agents.

The herbal mouthwash tested in this study consisted of Tvak taila (*Cinnamomum zeylanicum*), Pudina taila (*Mentha spicata*), Lavanga taila (*Syzigium aromaticum*), and Tailaparna taila (*Eucalyptus globulus*). The antimicrobial activity of cinnamon oil,[8] eucalyptus oil,[8] clove oil,[9] and mint oil[10] has been studied, with an anti-inflammatory action,[11-14] which suggests the role of these herbal agents in the prevention and treatment of periodontal disease.

Thus, the aim of this study was to evaluate the efficacy of a commercially available herbal mouthwash, which

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contains cinnamon oil (0.05% w/v), mint oil (0.05% w/v), clove oil (0.05% w/v), and eucalyptus oil (0.05% w/v), as an antimicrobial and an anti-inflammatory agent.

MATERIALS AND METHODS

A total of 84 subjects (45 males and 39 females; age range 20 to 35 years) visiting the Department of Periodontics, SDM College of Dental Sciences & Hospital, Dharwad, India, were recruited. The selection criteria for the subjects were (1) subjects of 18 years and above, (2) subjects with mild-to-moderate gingivitis and a GI score of ≤ 2, and (3) subjects compliant with the terms of the study. The exclusion criteria included (1) history of oral prophylaxis in past 6 months, (2) systemic antibiotic therapy in previous 6 months, (3) pregnant and lactating women, (4) history of systemic diseases, (5) smokers, and (6) history of known intolerance to any ingredient of either of the mouthwashes. An ethical clearance was obtained from the Institutional Ethical Committee, and an informed written consent was obtained from all the subjects before their participation in the study.

STUDY DESIGN

The study was carried out in two parts—an *in vitro* and an *in vivo* part.

In vitro Study Design

The *in vitro* part was carried out to investigate the antimicrobial activity of the mouthwashes (test and control) by determining their MIC against commercially available ready strains of *S. mutans*, *L. acidophilus*, *P. gingivalis,* and *F. nucleatum* using broth dilution method according to the antimicrobial susceptibility testing protocols given in 2007.[15]

In vivo Study Design

The study was a double-blinded investigation. A single examiner, who was blinded to the mouthrinses, conducted the study, and the three mouthrinses were labeled as A, B, and C by another investigator. Sample A (negative control) was a placebo rinse of distilled water; sample B (positive control) was a commercially available 0.2% CHX rinse (Rexidine; Indoco Pharmaceuticals Pvt. Ltd, Mumbai, India), and sample C (test) was the herbal mouthrinse (Befresh; Sagar Pharmaceuticals, Bengaluru, India).

The subjects were divided into four groups of 21 each, based on the treatment to be provided as group I: subjects receiving only scaling and root planning (SRP), group II: subjects receiving SRP and a placebo mouthwash [distilled water], group III: subjects receiving SRP and CHX mouthwash [Rexidin®], and group IV: subjects receiving SRP and the herbal mouthwash [Befresh®].

Full-mouth examination for PI,[16] GI,[16] and mSBI[17] was done at baseline visit by a single examiner, following which all the subjects received full mouth SRP. They were then allocated to one of the four groups and prescribed the respective mouthwashes to which the examiner and the subjects were blinded. The subjects of groups II, III, and IV were advised to use 10 mL of the respective mouthwashes, for a period of 2 weeks for 1 minute twice a day, 30 minutes after brushing. The PI, GI, and the mSBI were recorded again on the 14th day recall visit.

STATISTICAL ANALYSIS

The statistical analysis was carried out using the Statistical Package for the Social Sciences software with $p \leq 0.05$. Wilcoxon signed rank test was used to test the difference between the time intervals (baseline and 14 days) for PI, GI, and mSBI. The Kruskal–Wallis' analysis of variance test, chi-squared test, and Mann–Whitney U-test were used for intergroup comparisons of PI, GI, and mSBI.

RESULTS

All the 84 participants completed the study. Both the mouthwashes were well tolerated without any reports of allergies or any other adverse effects. The *in vitro* study showed that the organisms tested (*S. mutans, L. acidophilus, P. gingivalis,* and *F. nucleatum*) were susceptible to both the mouthwashes at the concentrations tested ranging from 10^{-1} to 10^{-9}, suggesting an antibacterial activity, as shown in Table 1. There was a statistically significant difference for the PI, GI, and mSBI scores from baseline to 14th day for all the four groups, as shown in Table 2. The mean difference in PI and GI scores between baseline and 14 days, using Mann–Whitney U-test, showed a statistically significant difference between the groups, as shown in Table 3. For mSBI, the mean difference was not statistically significant.

DISCUSSION

A major breakthrough in the research for a chemical means to prevent disease was with the advent of CHX, which is still considered as the gold standard.[6] However, because of its side effects, other mouthrinses containing herbal agents began gaining popularity. The present study was carried out with the aim of evaluating the antimicrobial and anti-inflammatory efficacies of two commercially available mouthrinses over a period of 2 weeks. The mouthrinses tested were Rexidin®, Befresh®.

To evaluate the antimicrobial efficacy of both the mouthwashes, they were tested *in vitro* against four pathogens, namely, *S. mutans, L. acidophilus, P. gingivalis,* and *F. nucleatum*, of which *S. mutans* and *L. acidophilus*

Table 1: Antimicrobial activity of Befresh® and Rexidin® mouthwashes at various dilutions against the four pathogens

Serial dilutions	*100*	*50*	*25*	*12.5*	*6.25*	*3.125*	*1.5*	*0.75*	*0.37*	*0.18*
Befresh										
S. mutans	S	S	S	R	R	R	R	R	R	R
L. acidophillus	S	S	S	S	S	S	S	R	R	R
P. gingivalis	S	S	S	S	S	S	S	S	S	S
F. nucleatum	S	S	S	S	S	S	S	S	S	R
Rexidin										
S. mutans	S	S	S	S	S	R	R	R	R	R
L. acidophillus	S	S	S	S	S	S	S	S	R	R
P. gingivalis	S	S	S	S	S	S	S	S	S	S
F. nucleatum	S	S	S	S	S	S	S	S	S	S

S: Sensitive; R: Resistant

Table 2: Group-wise mean and SD of PI, GI, and mSBI at baseline and 14 days

	Mean		*SD*		
Group	*Baseline*	*14 days*	*Baseline*	*14 days*	*p-value*
PI					
I	1.05	0.94	0.45	0.44	<0.001*
II	1.06	0.94	0.50	0.46	<0.001*
III	1.14	0.73	0.38	0.36	<0.001*
IV	1.06	0.80	0.35	0.30	<0.001*
GI					
I	1.03	0.91	0.48	0.46	<0.001*
II	1.15	1.03	0.54	0.53	<0.001*
III	1.30	0.81	0.39	0.33	<0.001*
IV	1.14	0.87	0.43	0.37	<0.001*
mSBI					
I	0.97	0.85	0.46	0.45	<0.001*
II	1.08	0.99	0.52	0.52	<0.001*
III	1.24	0.78	0.37	0.34	<0.001*
IV	1.04	0.77	0.40	0.37	<0.001*

*Statistically significant; SD: Standard deviation

Table 3: Intergroup comparisons for the mean difference in PI and GI scores

Groups	*Mean reduction in plaque scores*	*Z-value*	*p-value*
PI			
I *vs* II	−0.009	−0.999	0.318
I *vs* III	−0.300	−5.105	<0.001*
I *vs* IV	−0.150	−3.459	0.001*
II *vs* III	−0.291	−5.023	<0.001*
II *vs* IV	−0.141	−3.298	0.001*
III *vs* IV	0.150	−3.197	0.001*
GI			
I *vs* II	−0.004	−1.241	0.215
I *vs* III	−0.374	−5.502	<0.001*
I *vs* IV	−0.159	−4.318	<0.001*
II *vs* III	−0.370	−5.559	<0.001*
II *vs* IV	−0.155	−4.563	<0.001*
III *vs* IV	0.216	−4.506	<0.001*

*Statistically significant

are Gram-positive organisms and *P. gingivalis* and *F. nucleatum* are Gram-negative organisms. The antimicrobial efficacy was assessed by determining the MIC of the mouthwashes against these pathogens using broth dilution method.[15] The serial dilutions of Befresh® showed antibacterial activity against the periopathogens *P. gingivalis* and *F. nucleatum*, comparable with that of CHX, thus indicating that the contents of Befresh® have a potential antimicrobial activity, which corroborate with the studies conducted by Fani and Kohanteb[8] and Ayoola et al.[18]

The use of CHX is recommended for short-term (2 weeks) and not for medium- or long-term use due to its adverse effects like extrinsic tooth staining.[19] Hence, a duration of 2 weeks was decided for the present study.

The clinical part of this study was to test the anti-inflammatory efficacy of Befresh® as an adjunct to SRP; the negative control groups received only SRP and SRP with a placebo, as SRP is considered as a standard treatment for periodontal diseases.[20] Subjects with a GI score of ≤2 were included so as to avoid any heterogeneity among the gingival conditions of the subjects. To assess the anti-inflammatory efficacy, all the clinical parameters (PI, GI, mSBI) were recorded at baseline and 14 days. As plaque is considered to be an etiological factor for the development of gingivitis, PI was used to assess the amount of plaque accumulated. Outcome variables for gingivitis studies should include a visual index of gingival inflammation and a separate or component index of gingival bleeding.[21] Hence, the GI was used to grade the amount of gingival inflammation. For monitoring individual patients, both for response to initial therapy and during maintenance, an mSBI with three bleeding scores is recommended in preference to dichotomous scoring of bleeding[22] and, hence, the same was used in the present study.

A statistically significant difference (p-value <0.001) in the mean PI, GI, and mSBI scores from baseline to 14 days was seen for all the four groups, which can be attributed to the treatment (SRP or SRP with mouthwash) provided for the respective groups. Intergroup comparisons for the mean reduction in PI and GI scores showed a statistically significant reduction in groups III and IV as compared

with groups I and II, which can be attributed to the additional benefit of the mouthwashes used as an adjunct. Group III showed more reduction as compared with group IV (p-value = 0.001), which is in accordance with a previously published study by Mankodi et al,[23] which concluded that CHX digluconate shows better plaque inhibition and resolution of gingivitis.

In the present study, the herbal mouthwash showed a potential antimicrobial, antiplaque, and anti-inflammatory efficacy. But, in comparison with CHX gluconate, it has proven to be less effective. These results are in accordance with a study conducted by Haffajee et al.[24] However, CHX rinsing can cause a number of local side effects including extrinsic tooth and tongue brown staining, taste disturbance, enhanced supragingival calculus formation and, less commonly, desquamation of the oral mucosa. These side effects limit its acceptability to users and the long-term use of CHX-containing mouthrinses. Also, interactions between CHX and sodium lauryl sulfate, a commonly used dentifrice ingredient, have been demonstrated *in vivo*, which result in an interference with CHX activity. On the contrary, the herbal mouthwash showed results comparable with that of CHX and due to its natural ingredients did not cause any side effects during the study period of 2 weeks. Further studies with longer duration are required to prove that the herbal mouthwash can serve as a good alternative.

CONCLUSION

Thus, within the limitations of this study, it could be concluded that CHX and the herbal mouthwash were effective as anti-inflammatory agents when used as an adjunct to SRP. The herbal mouthwash had effects comparable with that of 0.2% CHX and it can also be safely prescribed as an adjunct to SRP, in order to reduce the disturbing local side effects of CHX.

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