Evaluation of a Herbal Mouthwash (Befresh™) Vs. Chlorhexidine Mouthwash (Clohex Plus): A Prospective Clinical and Microbiological Study

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Abstract

Background: Herbal medicine is both preventive and promotive in its approach. They contain various naturally occuring active ingredients that offer a gentle and enduring way of restoring health in a most trustworthy and least harmful way. The current research was carried out to evaluate the clinical and microbiological efficacy of a herbal mouthwash (BEFRESH™) with chlorhexidine mouthwash (CLOHEX Plus™) in reducing plaque and gingivitis among patients.

Materials and Methods: Thirty patients who participated in the study were divided randomly into two groups using the coin toss method. Each group containing 15 patients was prescribed either herbal mouthwash or chlorhexidine. Turesky modification of Quigley-Hein plaque index and Loe and Silness gingival index were recorded at baseline, 1st revisit, 2nd revisit and 3rd revisit. The patients were asked to report any discomfort, alteration in taste or any other side effects. A qualitative analysis was also carried out via questionnaire and group discussion to evaluate the side effects of the mouthwashes and patient compliance factor.

Results: The mean score of plaque index (PI), gingival index (GI) and colony forming units (CFUs) had significant difference between the two groups at baseline and 1st revisit, with lower value in the chlorhexidine group. After switching over between the groups, this difference was reduced and the values were very comparable at the 2nd and 3rd revisits. No discomfort or adverse effects were reported by the patients.

Conclusion: The herbal mouthwash is equally effective to chlorhexidine in reducing plaque and gingivitis among patients. The patient compliance was better towards the herbal mouthwash.

Keywords: Plaque; Gingivitis; Oral Hygiene Methods; Herbal Mouthwash; Chlorhexidine

Introduction

Periodontal diseases are multifactorial in nature and various risk and susceptibility factors have been proposed to explain the onset and progression of the diseases [1]. Bacterial plaque is considered to be the primary etiologic factor in initiating periodontal disease in the form of gingival inflammation [2]. Various species of microorganisms are associated with the plaque and they co-exist in the form of a biofilm on the tooth surface.

Evaluation of a Herbal Mouthwash (Befresh™) Vs. Chlorhexidine Mouthwash (Clohex Plus): A Prospective Clinical and Microbiological Study

210

The most significant development in the history of dentistry has been the emergence of a philosophy and dental practice based on prevention. Thus, the emphasis is laid more on oral hygiene and plaque control methods. Regular plaque control helps in preventing and controlling the progression of the disease. Mechanical plaque control is considered to be the gold standard of periodontal therapy [3]. It includes toothbrush, interdental floss, interdental brushes and woodsticks [4].

However, mechanical plaque control does not always suffice completely as its efficacy is dependent on the dexterity and motivation level of the patient [3]. Thus, chemical agents are advocated as adjuncts to mechanical methods to augment the plaque control. Mouthwashes are most commonly used for chemical plaque control. A mouth wash is a medicated liquid which is held in the mouth and swished by the action of perioral musculature to eliminate the oral pathogens [5]. They provide a means of depositing an active material for slow release in the mouth. So, they have antiplaque effect for a long period of time [1].

Herbal medicine is both preventive and promotive in its approach. The naturally occurring active ingredients in these herbal products offer a gentle and enduring way of restoring health in a most trustworthy and least harmful way [6]. India is known for its rich source of natural herbal products which can be used both topically and systemically. But the usage over a wide range is limited due to scant product testing and slower action [3]. Herbal mouthwashes usually do not contain alcohol or any added sugars or preservatives. This fact gives the herbal mouthwashes an edge over chlorhexidine mouthwashes [6].

The herbal mouth wash used in this study is composed of cinnamon oil (0.05 %), spearmint oil (0.30%), clove oil (0.05%) and eucalyptus oil (0.05%). Cinnamon (*Cinnamonum zeylanicum*) has an antibacterial and antifungal action. It is an efficient natural essence for neutralizing halitosis. The spearmint oil is rich in menthol and acts as a febrifuge, local anaesthetic, powerful anodyne and antiseptic agent. The clove oil acts as a topical antiseptic, anaesthetic, antibacterial, antifungal, antiviral and spasmolytic agent. The eucalyptus oil contains 70 - 85% eucalyptol which helps neutralize halitosis. It has antiseptic properties and acts as an antimicrobial, antifungal and bactericidal agent. The cinnamon and clove oils are particularly active against *Eurotium*, *Aspergillus and Penicillium* genus of microorganisms. It is completely alcohol free and does not contain any added sugars or preservatives.

Chlorhexidine is a cationic bisbiguanide with a very broad antimicrobial spectrum. It is the most widely used over the counter mouth wash. It is effective in reducing gingival inflammation. It is considered to be the gold standard and the major advantage is its long substantivity which is of 12 hours [6]. It binds to soft and hard tissues in the mouth, enabling it to act over a long period after application. However, chlorhexidine has several side effects, such as staining and taste alteration, which limit its long-term use. Therefore, chlorhexidine is used as a positive control in the current clinical trial of a new mouth wash formulation (BEFRESH) and is considered to be the gold standard [6].

In the present study, we have evaluated the clinical and microbiological efficacy of a herbal mouthwash (BEFRESH™) with chlorhexidine mouthwash (CLOHEX Plus) and the patient compliance towards the mouthwashes.

Materials and Methods

Study Population: The study was a double blinded prospective clinical comparative trial, carried out at Rajarajeswari Dental College and Hospital, Bangalore, India. The study protocol was approved by the ethical committee of the institution. The trial was a single centre study conducted at the department of Periodontology. The patients were explained about the study and a written informed consent was obtained from all the patients prior to the intervention.

Study Design: A total of 34 patients were assessed initially for eligibility for the study. Out of which 4 patients were excluded (3 did not meet inclusion criteria and 1 was not willing to participate in the study). Thus, thirty patients were included in the study. They were divided into two groups randomly using the coin toss method - group A (BEFRESH) (test group) and group B (CLOHEX Plus) (control

Evaluation of a Herbal Mouthwash (Befresh™) Vs. Chlorhexidine Mouthwash (Clohex Plus): A Prospective Clinical and Microbiological Study

211

group). The patient and the statistician were blinded in the study. Decoding of the mouthwashes was done only at the end of the study.

The inclusion criteria for the study were – (a) Localized or generalized chronic gingivitis patients (b) Age range of 18-35 years (c) No other systemic problems (d) Patients willing to participate in the study. The exclusion criteria were – (a) Localized or generalized chronic periodontitis patients (b) pregnant and lactating women (c) Patients with systemic diseases (d) Patients who refuse to participate in the study.

At the baseline, standardized oral hygiene procedures were performed on all patients. The patients were provided with the mouthwashes of their respective group. Same set of oral hygiene maintenance instructions were given orally to the patients. The tooth brush and tooth paste were also kept similar among the patients. The patients were recalled after 21 days from baseline, for the 1st revisit. Then a wash out period of 2 weeks was given and at the end of this period, a cross over between the groups was done [7,8]. The patients were recalled for a 2nd revisit after the wash out period. Again after 21 days the patients were recalled for a 3rd revisit.

At each visit, clinical measurements were taken in the form of Turesky modification of Quigley-Hein plaque index and Loe and Silness gingival index. The plaque samples were also collected at each visit for microbiological analysis. For collection and storage of plaque samples, 5 mL eppendorf tubes were used. Bacterial peptone water was used as the medium for storage of plaque samples. A review about the mouthwashes was taken from the patients at the 1st and 3rd revisits.

All the plaque samples collected were subjected to microbiological analysis. The colony forming units (CFUs) were counted by growing the colonies on sheep blood agar and MacConkey chocolate agar plates. The colony counts were recorded at the end of 2nd day after inoculation of the samples on the culture plates.

Statistical Analysis

Statistical Software Package SPSS version 22 (IBM SPSS Statistics for Windows, version 22.0, IBM Corp., released in 2013) was used to perform statistical analysis. Independent student t test was used to compare the mean PI, GI and CFUs between the two groups at different time intervals. Student paired t test was used to compare the mean PI, GI, and CFUs between different time intervals within each study group. The level of significance [P-value] was set at $p < 0.05$.

Results

Table 1 shows the values of plaque index and gingival index at baseline and 1st revisit. Independent student t test was performed to compare the mean value of the indices at baseline and post intervention period between group A and group B. The test results revealed that there was no statistically significant difference demonstrated between group A and group B at baseline period for PI and GI.

Comparison of mean scores of PI and GI between 02 study groups at different time intervals using Independent student t test									
Time	**Parameter**	**Group**	**N**	**Mean**	**SD**	**S.E.M**	**Mean Diff**	**t**	**P-Value**
Baseline	PI	Group A	15	1.66	0.45	0.12	0.30	2.011	0.05†
		Group B	15	1.36	0.36	0.09			
	GI	Group A	15	1.35	0.44	0.11	0.11	0.824	0.42
		Group B	15	1.24	0.30	0.08			
1st Revisit	PI	Group A	15	1.41	0.35	0.09	0.23	2.066	0.04*
		Group B	15	1.17	0.27	0.07			
	GI	Group A	15	1.11	0.33	0.09	0.05	0.519	0.61
		Group B	15	1.06	0.22	0.06			

Table 1: Comparison of mean scores of PI and GI between 02 study groups at different time intervals using Independent student t test.

During the post intervention period, Group B showed statistically significant lesser mean PI score 1.17 ± 0.27 than Group A [1.41 ± 0.35] at P = 0.04. However, the mean GI score did not significantly differ between the 02 groups. Hence, from this tabular data, we can infer that group B was significantly effective in reducing the plaque score compared to group A.

Citation: Kavita Chandrasekaran., *et al*. "Evaluation of a Herbal Mouthwash (Befresh™) Vs. Chlorhexidine Mouthwash (Clohex Plus): A Prospective Clinical and Microbiological Study". *EC Microbiology* 7.6 (2017): 209-218.

Evaluation of a Herbal Mouthwash (Befresh™) Vs. Chlorhexidine Mouthwash (Clohex Plus): A Prospective Clinical and Microbiological Study

212

Table 2 shows the values of indices at 2nd and 3rd revisits. Independent student t test was done to compare the mean PI and GI at 2nd and 3rd revisit periods between group A and group B. During the cross over period, the 2nd revisit which served as baseline period, demonstrated a statistically significant lesser mean PI score 1.19 ± 0.23 in Group B compared to Group A [1.45 ± 0.34] at P = 0.02.

Comparison of mean scores of PI and GI between 02 study groups at different time intervals using Independent student t test									
Time	Parameter	Group	N	Mean	SD	S.E.M	Mean Diff	t	P-Value
2nd Revisit	PI	Group A	15	1.45	0.34	0.09	0.25	2.417	0.02*
		Group B	15	1.19	0.23	0.06			
	GI	Group A	15	1.21	0.28	0.07	0.12	1.488	0.15
		Group B	15	1.09	0.14	0.04			
3rd Revisit	PI	Group A	15	1.06	0.15	0.04	0.03	0.822	0.42
		Group B	15	1.03	0.06	0.02			
	GI	Group A	15	1.01	0.10	0.02	0.00	0.000	1.00
		Group B	15	1.01	0.12	0.03			

***Table 2:** Comparison of mean scores of PI and GI between 02 study groups at different time intervals using Independent student t test.*

The 3rd revisit period, which marked the post intervention time during the cross-over of the study groups, demonstrated no statistically significant difference in PI and GI between group A and group B. Hence, we can infer that switching over of the intervention between the study groups has reduced the difference in the plaque score between group A and group B, thus demonstrating that BERFRESH mouthwash is equally potent as clohex.

Table 3 shows mean values of CFUs at baseline and 1st revisit. Mann Whitney U Test was performed to compare the mean CFUs at baseline and post intervention period between group A and group B. The test results revealed that there was no statistically significant difference demonstrated between group A and group B at baseline and post intervention periods.

Comparison of mean CFUs between 02 study groups at different time intervals using Mann Whitney U test								
Time	Group	N	Mean	SD	Min	Max	Z	P-Value
Baseline	Group A	15	42940.0	48363.5	100	100000	-1.575	0.12
	Group B	15	16540.0	34126.3	100	10000		
1st Revisit	Group A	15	940.0	2533.4	100	100000	-0.954	0.34
	Group B	15	220.0	316.7	100	1000		

***Table 3:** Comparison of mean CFUs between 02 study groups at different time intervals using Mann Whitney U test.*

Table 4 shows means values of CFUs at 2nd and 3rd revisits. Mann Whitney U Test was done to compare the mean CFUs at 2nd and 3rd revisit periods period between group A and group B.

Evaluation of a Herbal Mouthwash (Befresh™) Vs. Chlorhexidine Mouthwash (Clohex Plus): A Prospective Clinical and Microbiological Study

213

Comparison of mean CFUs between 02 study groups at different time intervals using Mann Whitney U test								
Time	**Group**	**N**	**Mean**	**SD**	**Min**	**Max**	**Z**	**P-Value**
2nd Revisit	Group A	15	13000.0	24451.1	1000	100000	-2.716	0.007*
	Group B	15	2680.0	3801.2	100	1000		
3rd Revisit	Group A	15	280.0	372.6	100	10000	-0.482	0.63
	Group B	15	220.0	316.7	100	1000		

***Table 4:** Comparison of mean CFUs between 02 study groups at different time intervals using Mann Whitney U test.*

During the cross over period, the 2nd revisit which served as baseline period, demonstrated a statistically significant lesser mean CFU of 2680.0 ± 3801.2 in Group B compared to Group A [13000.0 ± 3801.2] at P=0.007.

The 3rd revisit period, which marked the post intervention time during the cross-over of the study groups, demonstrated no statistically significant difference in mean CFUs between group A and group B. Hence, we can infer that switching over of the intervention between the study groups has reduced the difference in the mean CFUs between group A and group B, thus demonstrating that BERFRESH mouthwash is equally potent as CLOHEX Plus mouthwash.

Table 5 shows the mean values of the indices at baseline and 1st revisit. Student paired t test was done to compare the mean PI and GI between baseline and post intervention period within group A and group B. The test results demonstrated a statistically significant lesser mean PI and GI scores in the post intervention period compared to the baseline period in both group A and group B at P < 0.001. Hence, we can infer that both BERFRESH and CLOHEX Plus is equally potent in significantly reducing the PI and GI scores during the initial phase of the study.

Comparison of mean scores of PI and GI between different time intervals within each study group using Student Paired t test									
Groups	**Parameter**	**Time**	**N**	**Mean**	**SD**	**S.E.M**	**Mean Diff**	**t**	**P-Value**
Group A	PI	BL	15	1.66	0.45	0.12	0.25	5.206	<0.001*
		1st Revisit	15	1.41	0.35	0.09			
	GI	BL	15	1.35	0.44	0.11	0.24	5.392	<0.001*
		1st Revisit	15	1.11	0.33	0.09			
Group B	PI	BL	15	1.36	0.36	0.09	0.19	4.802	<0.001*
		1st Revisit	15	1.17	0.27	0.07			
	GI	BL	15	1.24	0.30	0.08	0.18	4.731	<0.001*
		1st Revisit	15	1.06	0.22	0.06			

***Table 5:** Comparison of mean scores of PI and GI between different time intervals within each study group using Student Paired t test.*

Evaluation of a Herbal Mouthwash (Befresh™) Vs. Chlorhexidine Mouthwash (Clohex Plus): A Prospective Clinical and Microbiological Study

214

Table 6 shows the mean indices values at 2nd and 3rd revisits. During the cross over period, Student paired t test was done to compare the mean PI and GI between 2nd and 3rd revisit periods within group A and group B. The test results demonstrated a statistically significant lesser mean PI and GI scores in the 3rd revisit period compared to the baseline period [2nd revisit period] in both group A [PI at P<0.001, GI at P=0.003] and group B [PI at P = 0.005, GI at P = 0.04]. Hence, we can infer that both BERFRESH and CLOHEX Plus is equally potent in significantly reducing the PI and GI scores during the cross over phase of the study as well.

Comparison of mean scores of PI and GI between different time intervals within each study group using Student Paired t test									
Groups	Parameter	Time	N	Mean	SD	S.E.M	Mean Diff	t	P-Value
Group A	PI	2nd Revisit	15	1.45	0.34	0.09	0.39	5.301	< 0.001*
		3rd Revisit	15	1.06	0.15	0.04			
	GI	2nd Revisit	15	1.21	0.28	0.07	0.21	3.607	0.003*
		3rd Revisit	15	1.01	0.10	0.02			
Group B	PI	2nd Revisit	15	1.19	0.23	0.06	0.17	3.371	0.005*
		3rd Revisit	15	1.03	0.06	0.02			
	GI	2nd Revisit	15	1.09	0.14	0.04	0.09	2.229	0.04*
		3rd Revisit	15	1.01	0.12	0.03			

Table 6: *Comparison of mean scores of PI and GI between different time intervals within each study group using Student Paired t test.*

Table 7 shows the mean CFUs at baseline and 1st revisit. Wilcoxon Signed Rank test was done to compare the mean CFUs between baseline and post intervention period within group A and group B. The test results demonstrated a statistically significant lesser mean CFUs scores in the post intervention period compared to the baseline period in both group A and group B at P = 0.001. Hence, we can infer that both BERFRESH and CLOHEX Plus is equally potent in significantly reducing the mean CFUs scores during the initial phase of the study.

Comparison of mean CFUs between different time intervals within each study group using Wilcoxon Signed Rank Test								
Groups	Time	N	Mean	SD	Min	Max	Z	P-Value
Group A	Baseline	15	42940.0	48363.5	100	100000	-3.311	0.001*
	1st Revisit	15	940.0	2533.4	100	100000		
Group B	Baseline	15	16540.0	34126.3	100	10000	-3.462	0.001*
	1st Revisit	15	220.0	316.7	100	1000		

Table 7: *Comparison of mean CFUs between different time intervals within each study group using Wilcoxon Signed Rank Test.*

Table 8 shows mean CFUs at 2nd and 3rd revisits. Wilcoxon Signed Rank test was done to compare the mean CFUs between 2nd and 3rd revisit periods within group A and group B. The test results demonstrated a statistically significant lesser mean CFUs scores in the post intervention period [3rd revisit] compared to the baseline period [2nd revisit] in both group A and group B at P = 0.001. Hence, we can infer that both BERFRESH and CLOHEX Plus is equally potent in significantly reducing the mean CFUs scores during the cross over phase of the study as well.

Evaluation of a Herbal Mouthwash (Befresh™) Vs. Chlorhexidine Mouthwash (Clohex Plus): A Prospective Clinical and Microbiological Study

215

Comparison of mean CFUs between different time intervals within each study group using Wilcoxon Signed Rank Test								
Groups	**Time**	**N**	**Mean**	**SD**	**Min**	**Max**	**Z**	**P-Value**
Group A	2nd Revisit	15	13000.0	24451.1	1000	100000	-3.376	0.001*
	3rd Revisit	15	280.0	372.6	100	10000		
Group B	2nd Revisit	15	2680.0	3801.2	100	1000	-3.354	0.001*
	3rd Revisit	15	220.0	316.7	100	1000		

Table 8: Comparison of mean CFUs between different time intervals within each study group using Wilcoxon Signed Rank Test.
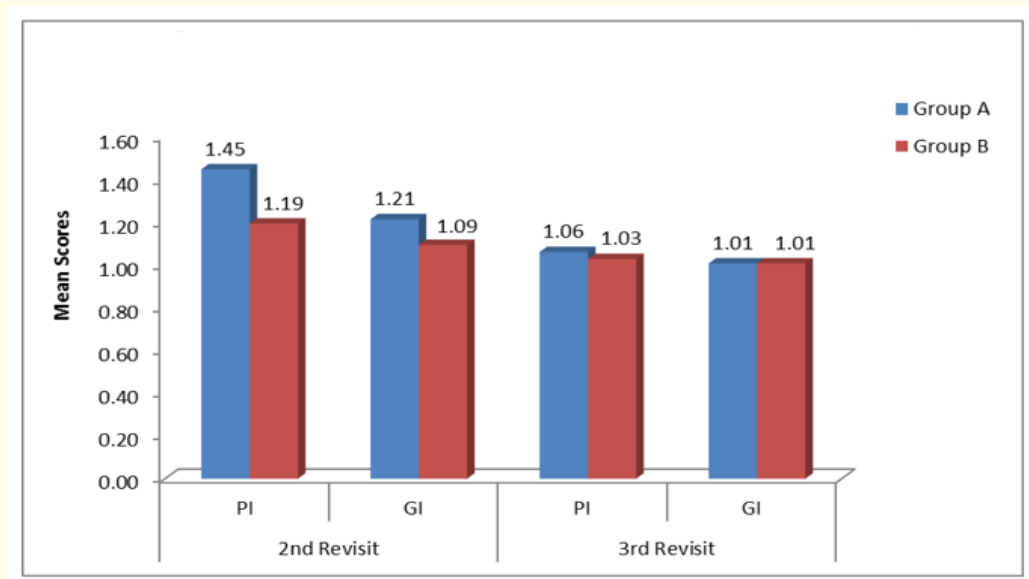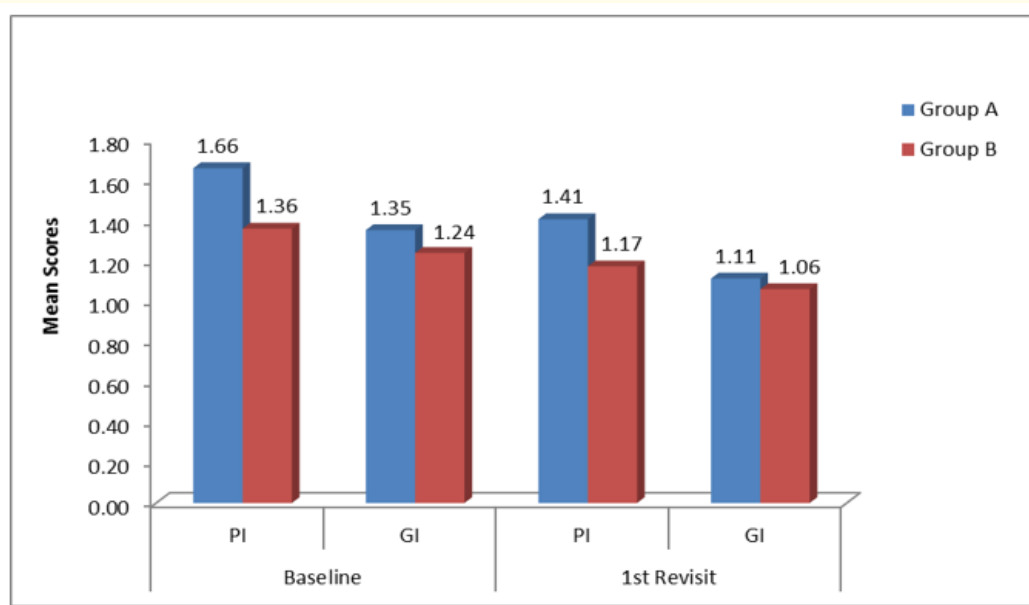

Figure 1 and 2: Comparison of mean scores of PI & GI between 02 study groups at different time intervals.
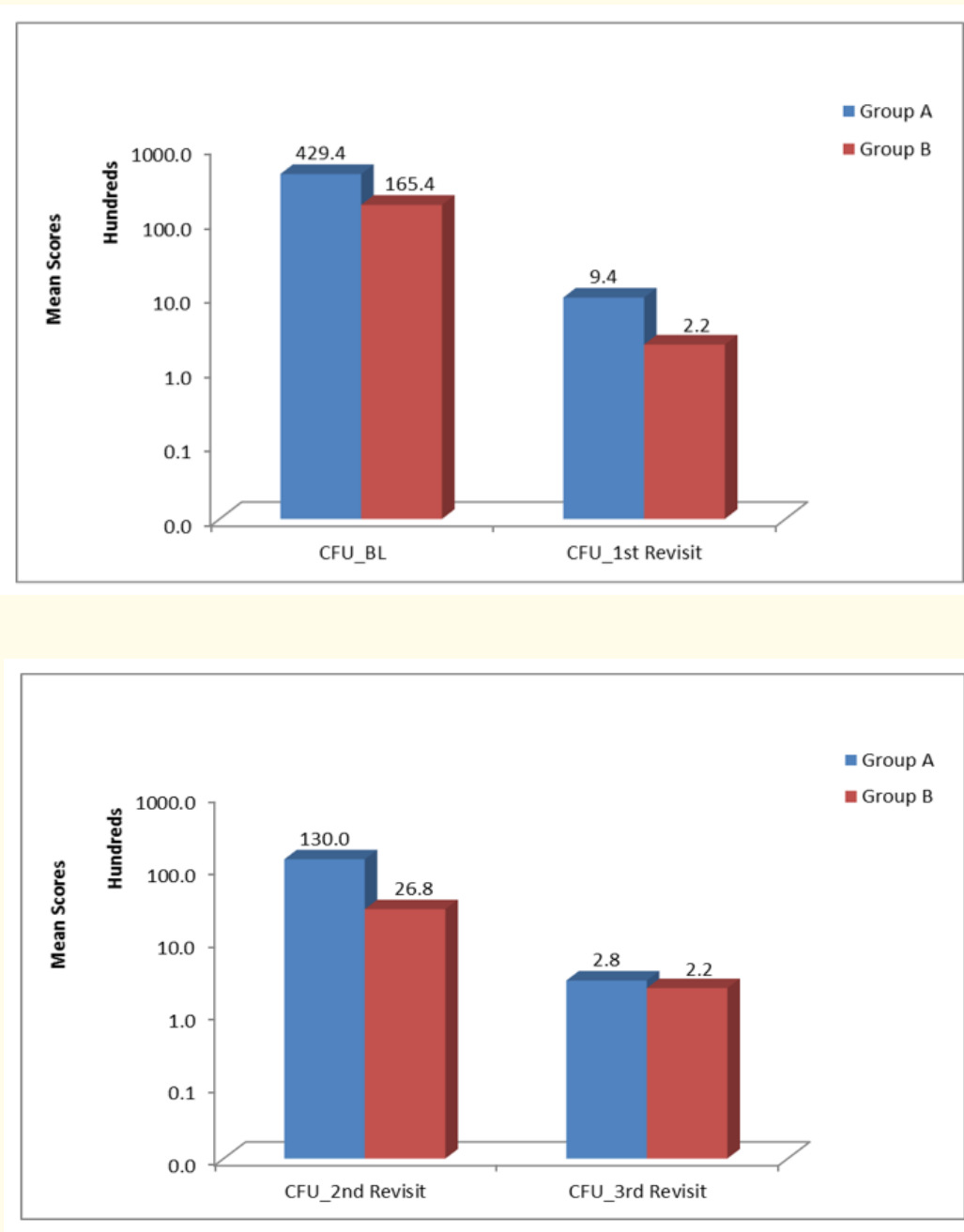
Evaluation of a Herbal Mouthwash (Befresh™) Vs. Chlorhexidine Mouthwash (Clohex Plus): A Prospective Clinical and Microbiological Study

216

Figure 3 and 4: Comparision of mean CFUs between 02 study groups at different time intervals.

Discussion

Herbal medications have been introduced as an alternative medicine to prevent and treat oral conditions. A widespread use of such

Evaluation of a Herbal Mouthwash (Befresh™) Vs. Chlorhexidine Mouthwash (Clohex Plus): A Prospective Clinical and Microbiological Study

217

products among the population will be beneficial to even the low socioeconomic status patients. These products are cost effective and have shown to pose minimal or no side effects. The current study has attempted to show the efficacy of one such herbal product called BERFRESH in comparison to the gold standard of mouthwashes viz. chlorhexidine.

The results of the current study have shown that the mean scores of plaque index, gingival index and colony forming units (CFUs) recorded at baseline and at 1st revisit were consistently lower in the group B (chlorhexidine) with the a significant difference from the value of group A (Befresh). After the cross over between the groups, these values were recorded at 2nd and 3rd revisits and they were comparable with no significant difference. These results have been shown in the tables (Table 1-8) and graphs (Figure 1-4).

These results were comparable with few other studies conducted earlier. A study conducted by Aspalli S., *et al*. in 2013, showed that a herbal mouthwash is effective in treatment of plaque induced gingivitis, it has lesser side effects and it can be effectively used as an adjunct to mechanical therapy [10]. A study by Kothiwale SV., *et al*. conducted in 2014, showed that a herbal mouthwash is clinically beneficial as antiplaque, antigingivitis and antimicrobial agent and can be used as a regular mouth wash [1]. A study carried out by Vijayalakshmi LG, Geetha RV., in 2015, proved that the herbal mouthwash used in the study had antimicrobial activity equal to the activity of conventional chlorhexidine mouth wash [11]. Bagchi S., *et al*. in 2015, showed in their study that chlorhexidine group is the best anti plaque and antigingivitis agent, but herbal mouth wash also showed gradual improvement from baseline to 21 days [4]. A study by Priya BM., *et al.* in 2015, showed that the green tea containing mouthwash used in the study was equally effective to chlorhexidine in reducing gingival inflammation and plaque [3].

A few other studies were conducted earlier which did not show similar results. A study by Nagappan., *et al*. in 2012, showed that compared to herbal mouth rinse, chlorhexidine mouth rinse provided better results in its antimicrobial efficacy against Streptococcus mutans [6]. A study by Parwani SR., *et al*. 2013 showed that 0.2% chlorhexidine gluconate mouthwash remains the best anti-plaque agent [12]. Weijdan FAV., *et al*. 2015 showed in their study that mouthwash containing chlorhexidine (CHX) is the first choice. The most reliable alternative for plaque control can be essential oil (EO). There is no difference between CHX and EO with respect to gingivitis was observed [13]. Nagappan N., *et al*. 2016 showed in their study that chlorhexidine mouthrinse (0.2%) has a better antimicrobial efficacy against the *S. mutans* when compared to herbal mouthrinse used in the study (arowash liquid) [14].

Conclusion

The positive results of the study have led to the conclusion that the newly formulated herbal essential oil mouth wash (Befresh) is equivalent in efficacy to chlorhexidine in reducing plaque and gingival inflammation among patients. Also, the patient compliance was better towards the herbal mouthwash as no side effects were reported by the patients for it when compared to chlorhexidine.

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Evaluation of a Herbal Mouthwash (Befresh™) Vs. Chlorhexidine Mouthwash (Clohex Plus): A Prospective Clinical and Microbiological Study

218

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