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EFFECTIVENESS OF HERBAL AND 0.12% CHLORHEXIDINE MOUTHWASH ON GINGIVITIS AND SALIVARY NEUTROPHIL COUNTS

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ABSTRACT

Background and Aim: Use of mouthwashes as an adjunct in the daily oral hygiene regimen has been strongly recommended by the dentists worldwide. Chlorhexidine has in fact been proven to be a gold standard in mouthwashes and is known to significantly reduce/prevent gingivitis. But owing to its side-effects of tooth discoloration and transient taste alteration on its long term use, there has been a constant effort to discover an equally efficient mouthwash with minimal or no side-effects. The aim of this study was to evaluate the effectiveness of a commercially available herbal mouthwash (Himalaya HiOra Regular) in comparison to 0.12% Chlorhexidine mouthwash in reducing gingivitis and salivary neutrophil count. **Methods and Materials:** This study was a double blinded, randomized design. The herbal mouthwash (Himalaya HiOra Regular) was compared to 0.12% Chlorhexidine mouthwash (positive control).The patients gingival health status was evaluated using Gingival Index and Salivary Neutrophil Count (using Fluorescent Microscope) at baseline visit, 15th day and 30th day of intervention. **Results:** Out of the 36 subjects randomized, none were lost to follow up and a total of 18 were analysed in Group A(0.12% Chlorhexidine) and 18 in Group B(Himalaya HiOra Regular Mouthwash). Both the groups showed a significant reduction in Gingival Index Score (Group A – 17.27% & Group B – 13.25%) and in Salivary Neutrophil Counts (Group A – 35.38% & Group B – 32.12%). There was no significant difference in between the two groups both in the Gingival Scores ($p>0.05$) and in the salivary neutrophil counts ($p>0.05$). **Conclusions:** This study has shown that herbal mouthwash is at par with the gold standard in reducing gingivitis in a 4 week period. Thus herbal mouthwash forms a promising adjunct to the daily oral hygiene regimen with no noted side effects reported.

KEYWORDS: Herbal, HiOra, Chlorhexidine, Neutrophils, Fluorescent Microscope, Gingivitis.

INTRODUCTION

Dental caries and periodontal diseases account for the most important global oral health burdens. The presence of microbial plaque on the tooth surfaces and at the gingival margin plays a vital role in the development of these easily preventable oral diseases. Thus, the prevention and treatment of most prevalent oral diseases like dental caries and periodontal diseases focuses mainly on the supragingival plaque control. Mechanical removal of plaque using toothbrush has been proven to be an efficient means of plaque debridement and thereby helps in improving/maintaining good oral health.[1] On the contrary, several researches have also concluded that mechanical control of plaque is not sufficient to prevent or cure gingival/periodontal diseases.[2-4] The most common reason given to explain this is the limited cleaning in the interdental areas by mechanical cleaning aids. Inability of patients to properly brush their teeth due to any physical or mental handicap also accounts for inefficiency of mechanical plaque removal.[5-7] Other oral hygiene measures like use of mouth rinse and dental floss that removes plaque and prevents its accumulation on teeth and gingiva act as essential adjuncts for maintaining good oral health.[8] Of the various adjuncts that were developed and whose effectiveness was clinically tested, the most extensively studied is Chlorhexidine mouth wash. In the last 30 years, many randomized controlled trials have ascertained the effectiveness of chlorhexidine and thus have recognized this chemical plaque controlling agent as a gold standard.[9-14] Due to the most common side effects reported for chlorhexidine of tooth discoloration and altered taste sensation[15,16] its long term use is contra-indicated. Non chlorhexidine mouthwashes such as those containing essential oil (like Listerine) are also available

but with reduced efficacy as compared to the gold standard.

There is a growing interest in research of a complementary and alternative remedy that not only cures gingival inflammation but that can also act as an effective adjunct in the daily oral hygiene regimen with minimal/no side-effects.

The aim of this study was to evaluate and compare the effectiveness of a commercially available herbal mouthwash (Himalaya HiOra Regular) with 0.12% Chlorhexidine on Gingival inflammation and salivary neutrophil count over a 4 week period.

METHODS

Study Design and Population

This two-celled, double-blinded parallel randomized controlled trial was carried out in the Department of Public health Dentistry, S.D.M. College of Dental Sciences and Hospital, Dharwad, Karnataka, India. Inclusion Criteria for this study was males and females aged 18-49 years having gingival inflammation with a Gingival Index (Modified Loe and Silness Index) Score of more than 1 and who willingly signed the informed consent form. Exclusion criteria for this study was subjects suffering from any systemic conditions like diabetes, hypertension, cardiac problems, pregnant or lactating women, subjects undergoing orthodontic treatment, subjects who are administering or who have in the past 4 weeks administered antibiotics for any reason and subjects who have a habit of smoking, alcohol or drug abuse.

Eligible subjects were randomized to receive either a commercially available herbal mouthwash – HiOra Regular manufactured by Himalaya Co. or a 0.12% Chlorhexidine mouthwash. The randomization process was made externally by a separate investigator (other than the principal investigator and examiner) following the principle of minimization. Through this randomization it is ascertained that both the groups have the subjects so distributed that the mean Gingival Index Scores and Age in both the groups is similar with no statistical significant difference. The examiners measuring the clinical status of the subjects by GI Index and Salivary Neutrophil scoring were neither involved in the randomization process nor were they aware of the assigned products. Subjects codes was printed on sealed bottles containing either the experimental or the control dentifrices and each subject had to use only the mouth rinse bottle assigned during the follow-up. The appearance of the two mouth rinses were identical (Mouthwashes were overwrapped to hide their identity). The final protocol of the study was approved by the Ethical Committee of S.D.M. College of Dental Sciences and Hospital, Dharwad, Karnataka, India (SDMCDSH-IEC) IRB No. 2017/P/CMM/44.

Sample Size Estimation

The sample size was calculated using G Power Software. The mean values of the two studies were considered from a study conducted earlier.[17] The calculated sample size in each group was 13 in each group. Keeping into account 25% regression rate, the sample size was extrapolated to 18 in each group. (N=36; N_A=18 & N_B=18).

METHODOLOGY

For the purpose of standardization, all the subjects fulfilling the inclusion criteria and who willingly signed the consent form were given the same brand toothpaste (Colgate Cavity Protection) and medium bristled tooth brush. The subjects were instructed to use only the given tooth paste and tooth brush following the Modified Bass Technique for a *washout* period of 7 days and return for the baseline visit on the 8th day. The subjects were asked to come for the baseline evaluation early morning without brushing/rinsing/eating or even drinking. As the subjects arrived, their salivary sample was collected by asking them to rinse their mouth thoroughly with 10mL of 0.9% Normal Saline for 30 seconds and expectorate the rinse into the test tubes (labeled with their respective subjects codes) provided. The sample thus collected was vertexed for 20 seconds and was processed for salivary neutrophil count using a fluorescent microscope. The subjects were then examined for gingival inflammation using the modified Loe and Silness Gingival Index. The subjects were randomized to either of the two groups based on their baseline GI scores. The subjects in both the groups were instructed to use only the given tooth paste and tooth brush (same as used for the washout period) twice a day. After brushing, the subjects were asked to take 10mL of the respective allotted product and rinse their mouth thoroughly for 30 seconds. The subjects were informed to come for re-evaluation at 15th day and 30th day.

STATISTICAL ANALYSIS

The data was entered into the computer (MS-Office 2007, Excel data sheet).The data was subjected to statistical analysis using the statistical package (SPSS version 20.0). Statistical significance was recorded at **p-value less than 0.05**.

Shapiro-Wilk test was done to assess Normality. Since the majority of the variables did not have normal distribution ($p<0.05$), non-parametric tests were applied. The gingival scores and salivary neutrophils count were compared with in each group by Friedman's Test. In between the two groups, Mann Whitney U Test was performed at Baseline, 15th day and 30th day visit with respect to their gingival scores and salivary neutrophils count.

RESULTS

A total of 36 subjects full-filling the inclusion criteria and who signed the informed consent were enrolled into the study. The selected subjects were given a

commercially available fluoride tooth paste (Colgate Cavity Protection) for a wash out period of 7 days. At the baseline visit, the subjects were assessed for their gingival status and salivary neutrophil count. They were randomly allocated into the two groups based on their Loe and Silness Gingival Index scores (Primary Outcome Variable).

The subjects were re-evaluated for gingival status and salivary neutrophil count on the 15th day and 30th day post intervention. All the subjects recruited in the study completed the study and were subjected to statistical analysis (Fig. 1).

There was no statistically significant difference in mean age between the groups (27 and 26.44 years, respectively). At baseline, no significant differences were detected among the two groups with respect to mean gingival index.

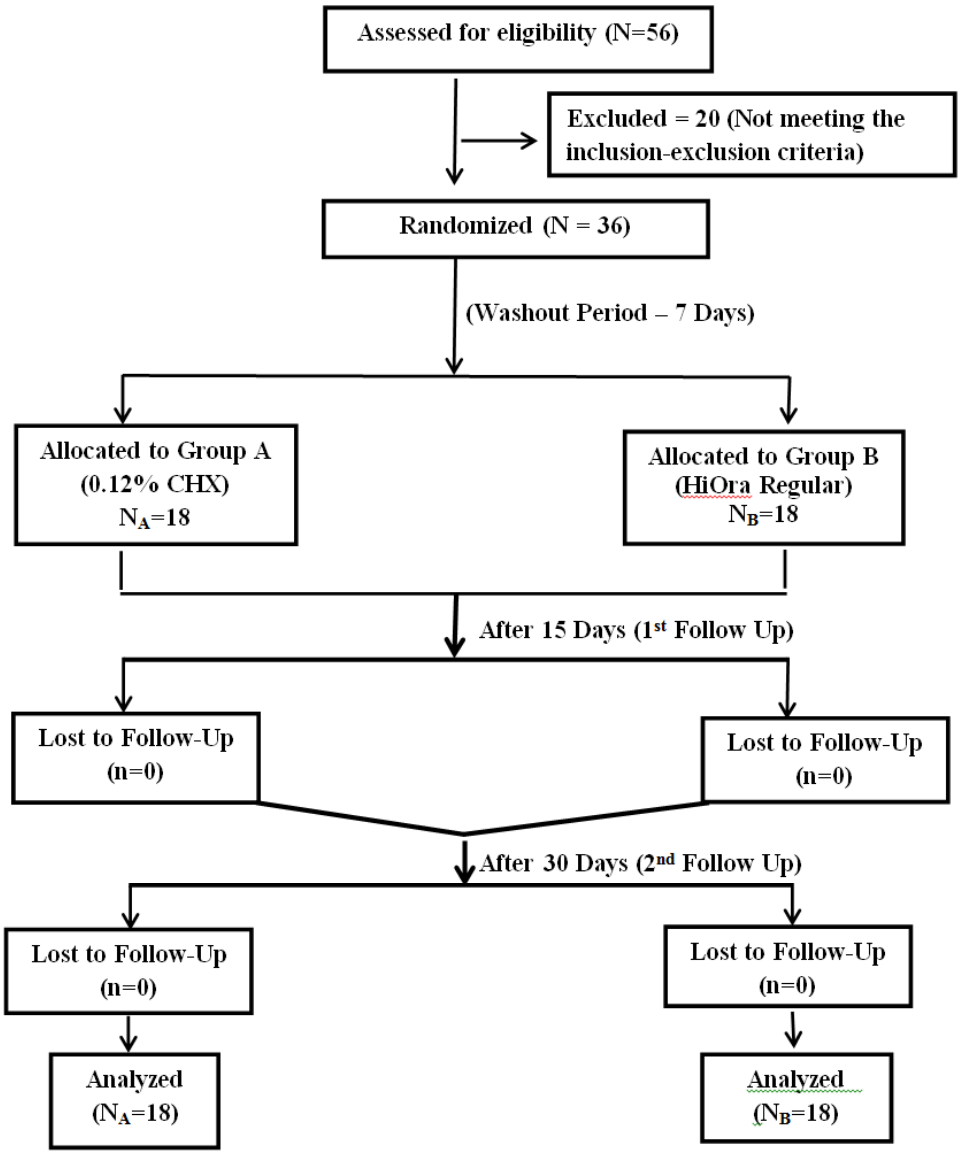

Figure. 1: Consort Flow Diagram showing the distribution of the study subjects through each stage of the trial.

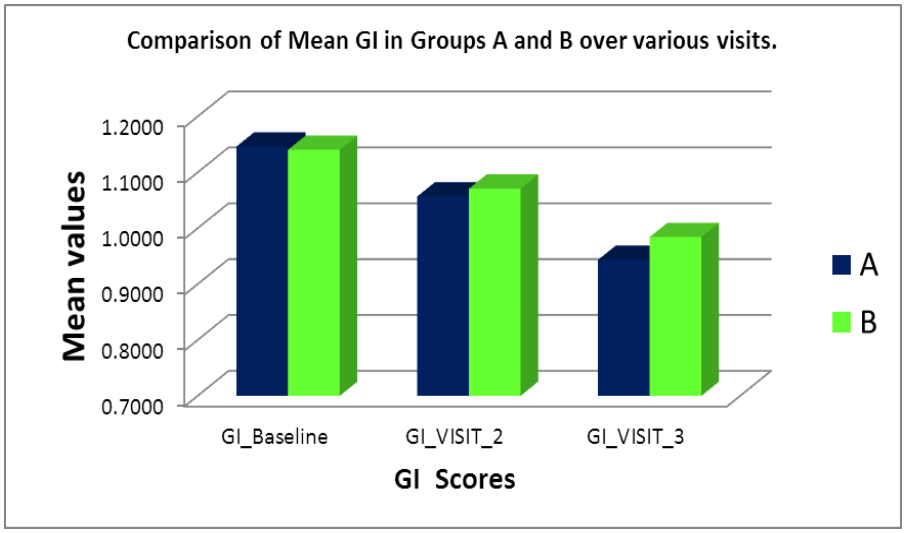

Figure. 2: Comparison of Mean GI values over different visits for groups A and B.

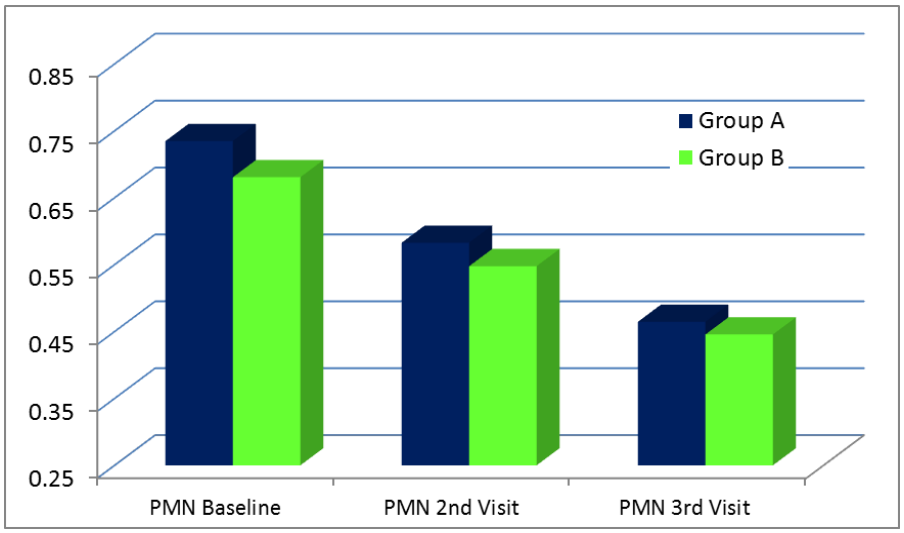

Figure. 3: Comparison of Mean PMN values over different visits for groups A and B.

Table. 1: Comparison of Gingival index (GI) scores at baseline, 2nd visit and 3rd visit among the patients in Group A (0.12% CHX) and Group B (HiOra Regular) using Friedman test.

		N	Mean	Std. Deviation	Test Statistic	P Value
A	GI Baseline	18	1.15433	.171194	35.271	<0.0001
	GI-2nd Visit	18	1.06667	.138776		
	GI-3rd Visit	18	.95133	.122649		
	PMN-Baseline	18	.76833	.455709	36	<0.0001
	PMN-2nd visit	18	.60833	.369411		
	PMN-3rd visit	18	.48417	.296684		
B	GI Baseline	18	1.14050	.104587	36	<0.0001
	GI-2nd Visit	18	1.07083	.094250		
	GI-3rd Visit	18	.98422	.092048		
	PMN-Baseline	18	.62361	.240898	36	<0.0001
	PMN-2nd visit	18	.51056	.205268		
	PMN-3rd visit	18	.42556	.179459		

Table. 2: Wilcoxan Signed Ranks post hoc test in Group A and Group B. Test Statistics[a].

Product		GI-2nd Visit - GI Baseline	GI-3rd Visit - GI Baseline	GI-3rd Visit - GI-2nd Visit	PMN-2nd visit - PMN-Baseline	PMN-3rd visit - PMN-2nd visit	PMN-3rd visit - PMN-Baseline
A	Z	-3.624[b]	-3.724[b]	-3.725[b]	-3.724[b]	-3.725[b]	-3.724[b]
	Asymp. Sig. (2-tailed)	.000	.000	.000	.000	.000	.000
B	Z	-3.726[b]	-3.724[b]	-3.725[b]	-3.725[b]	-3.728[b]	-3.725[b]
	Asymp. Sig. (2-tailed)	.000	.000	.000	.000	.000	.000
a. Wilcoxon Signed Ranks Test							
b. Based on positive ranks.							

Group A: The mean gingival scores in Group A (0.12% CHX) observed at Baseline, 15th Day and 30th Day in Group A was 1.154±0.10, 1.067±0.08 and 0.951±0.08 respectively**.** Friedman Test showed that there was a highly statistically significant difference between the three visits in the gingival scores ($p<0.01$). Wilcoxan Post hoc test showed a significant difference was noticed ($p<0.05$) in between baseline-1st visit, baseline-2nd visit and 1st-2nd visit.

The mean salivary neutrophils count (in lacs per mm^3 of saliva) observed at Baseline, 15th Day and 30th Day was 0.768±0.03, 0.68±0.04 and 0.484±0.03 respectively**.** Friedman Test showed that there was a highly statistically significant difference between the baseline visit and 15th day visit, baseline to 30th day visit in the gingival scores ($p<0.01$). Wilcoxan Post hoc test showed a significant difference was noticed ($p<0.05$) in between baseline-1st visit, baseline-2nd visit and 1st-2nd visit.

Group B: The mean gingival scores in Group B (HiOra Regular) observed at Baseline, 15th Day and 30th Day was 1.141±0.10, 1.071±0.10 and 0.984±0.07 respectively**.** Friedman Test showed that there was a highly statistically significant difference between the baseline visit and 15th day visit, baseline to 30th day visit in the gingival scores ($p<0.01$). Wilcoxan Post hoc test showed a significant difference was noticed ($p<0.05$) in between baseline-1st visit, baseline-2nd visit and 1st-2nd visit. The mean neutrophil scores in Group B (HiOra Regular) (in lacs per mm^3 of saliva) observed at Baseline, 15th Day and 30th Day was 0.624±0.05, 0.511±0.03 and 0.426±0.02 respectively**.** Test showed that there was a highly statistically significant difference between the baseline visit and 15th day visit, baseline to 30th day visit in the gingival scores ($p<0.01$). Wilcoxan Post hoc test showed a significant difference was noticed ($p<0.05$) in between baseline-1st visit, baseline-2nd visit and 1st-2nd visit.

Table. 2. Comparison of mean gingival scores and mean PMN scores in between Group A (0.12% CHX) and Group B (HiOra Regular) using Mann Whitney U Test.

	NULL HYPOTHESIS	Sig.	Decision
1.	The distribution of GI_Baseline is the same across Group A and Group B.	.620	Retain the Null Hypothesis
2.	The distribution of GI – 1st Visit is the same across Group A and Group B.	.461	Retain the Null Hypothesis
3.	The distribution of GI – 2nd Visit is the same across Group A and Group B.	.343	Retain the Null Hypothesis
4.	The distribution of PMN – Baseline Visit is the same across Group A and Group B.	.893	Retain the Null Hypothesis
5.	The distribution of PMN – 1st Visit is the same across Group A and Group B.	.869	Retain the Null Hypothesis
6.	The distribution of PMN – 2nd Visit is the same across Group A and Group B.	.599	Retain the Null Hypothesis

The significance level set at the 0.05.

Mann Whitney U Test showed that there was no statistically significant ($p>0.05$) difference between Group A and Group B in the GI scores and PMN scores at baseline, 1st visit and 2nd visit.

DISCUSSION

Mechanical plaque removal using toothbrush and a dentifrice is undoubtedly the primary method to prevent dental diseases. However, a more intensive understanding of the nature of dental diseases has dramatically rejuvenated interest in chemical methods of plaque control.[18]

Years of documented research have established that chlorhexidine digluconate, a **gold standard** among mouthwashes, are safe, stable and owing to its great substantivity are highly effective in preventing and controlling plaque formation thus curing and even preventing the development of gingivitis.[19]

Yet, its long term use is contraindicated due to its side effects which demand the search of equally effective alternatives that have minimal or no side-effects. Nowadays, patients are more interested in CAM - complementary and alternative medicines - to cure and

prevent diseases owing to the lack of any documented side-effects of these herbal alternatives. But due to lack of scientific evidence, doctors are not able to recommend the use of such Herbal products. Thus this research was undertaken to bring about a scientific evidence whether or not to recommend herbal mouthwash for the treatment of gingival inflammation.

A randomized double blinded controlled pilot study conducted by Shetty et al to assess and the clinical and microbiological efficacy of chlorhexidine and a herbal mouthrinse in patients with chronic gingival inflammation[20] showed no statistically significant differences between the two groups with regard to the clinical parameters and colony counts of the bacteria. This is in accordance with the present study as per the Gingival Index parameter is considered.

Another study[21] conducted to assess and compare the de novo plaque formation between Triphala, Hiora and Chlorhexidine also concluded that there were no statistically significant differences between the two groups with regard to the clinical parameters and colony counts of the bacteria.

The anti-plaque and anti-gingivitis properties have been shown to be the best at 0.2% concentration of Chlorhexidine. But due to the strong taste and complain of burning sensation, this concentration often leads to poor patient compliance. A three month clinical trial conducted by Segreto et al to compare the effects of 0.2% and 0.12% of chlorhexidine mouthrinse on gingivitis showed that a 0.12% chlorhexidine mouthrinse provided the same clinical benefits as a 0.20% chlorhexidine mouthrinse when used under a twice daily regimen.[22] Thus in this study 0.12% of Chlorhexidine was taken as a positive control group.

Taking into consideration that all other methods suggested to be included in the oral hygiene regimen can only be an adjunct to the mechanical plaque removal, the ADA demands an evaluation period of at least 4 weeks to test the efficacy of such products in clinical trials (American Dental Association 1997, 2008). Thus, a follow up period of 4 weeks was taken.[6]

The Himalaya Co. Ltd. Manufactures a wide range of herbal products that are trusted by the consumers worldwide. They advocate the daily use of HiOra Regular Mouth wash in order to prevent/cure gum diseases. There is lack of sufficient scientific evidence in support of this easily available and cost effective herbal mouthwash. Therefore, in this study, HiOra Regular Mouthwash was selected.

The study was conducted among the adult population of Hubli- Dharwad city aged between 18-49 years, who fulfilled the inclusion and exclusion criteria and who signed the informed consent form. This age group was chosen since mild to moderate forms of gingivitis occur more commonly in this age group and signs of advanced periodontal diseases like mobility, pockets, recession, etc are common in those aged above 45 years.

The examiner was blinded in the present study to eliminate bias. All examinations were performed by a single examiner. The assignment of subjects to groups was done by a person other than the chief investigator, who also dispensed the products and provided instructions to all the study participants. The products were concealed properly with labeling of 'A' and 'B' written over the 0.12% CHX bottle and HiOra Regular mouthwash bottle respectively. This ensured blinding of the subjects. However, the dispensing of the products and provision of the instructions were undertaken in a place that was away from the chief investigator, who was examining the subjects for plaque and gingivitis scores. This ensures that the chief investigator and the recorder were not aware as to which group the study subjects were allotted to.

A 7 day period was regarded as the **washout period** and was expected to remove any carry over effect which might be there due to the use of their oral hygiene products before being enrolled into this study. According to the investigators, the risk of neutrophils from the Gingival Crevicular Fluid or saliva being washed away will be minimized thus helping in the true quantification of salivary PMNs.

Salivary bio-markers is a growing area of research in the early diagnosis and prognostic variable for various diseases. PMNs act as the first line of defense in the body, yielding an inflammatory response to curb down the foreign substances. Salivary neutrophils directly correspond to the periodontal disease and treatment response. Thus, a single rapid salivary rinse can serve as an excellent research tool for quantifying the gingival inflammation.[23] Salivary neutrophil quantification was done under Fluorescent Microscope, using Acridine Orange Dye as the cells are very well stained under this dye making it very easy to appreciate its count under the fluorescent microscope.

The gingival inflammation was assessed according to the Loe and Silness gingival index (modified) since it is the most widely accepted and used gingival index due to its documented validity, reliability and ease of use.

The daily oral hygiene procedures were not supervised. However, reinforcement regarding the use of oral hygiene products was provided every week. The compliance of study subjects were monitored at the follow up visits by measuring the amount of tooth pastes and mouth rinses left over. Also, throughout the course of the investigation the compliance was further monitored and reinforced with phone calls to each subject between examination visits by a person other than the chief investigator.

All studies in which oral hygiene is permitted suffer from the drawback of the Hawthrone effect, with all participants tending to improve their behavior because of their participation in a research project. Although it is expected that this effect becomes smaller over time, one cannot exclude the possibility of this effect influencing the study outcomes, which could have played a role in the present study as well.

Even though there were no noted side effects of the herbal (HiOra Regular) mouthwash, long term follow- up is required to evidently conclude the long term usage safety of the product.

CONCLUSION

Within the limitations of the present study - HiOra Regular (Herbal) Mouthwash and 0.12% Chlorhexidine – both are seen as an effective adjunct to manual tooth brushing in reducing plaque induced gingivitis in a 30 day period. There is no statistical difference noted in the effectiveness of the two products in reducing gingival inflammation and salivary neutrophil counts over a 30 day period.

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CoNFLICT OF INTEREST AND SOURCE OF FUNDING

All authors declare that they have no conflicts of interest. Himalaya Co. provided the materials (Himalaya HiOra Mouthwash) for this study but had no role in the planning, conduction, data analysis or in any other phase of this study

REFERENCES

1. Van der Weijden GA, Hioe KP. A systematic review of the effectiveness of self-performed mechanical plaque removal in adults with gingivitis using a manual toothbrush. J Clin Periodontol, 2005 Oct 1; 32(s6): 214-28.
2. Lang WP, Ronis DL, Farghaly MM. Preventive behaviors as correlates of periodontal health status. J Public Health Dent., 1995 Jan 1; 55(1): 10-7.
3. Stewart JE, Strack S, Graves P. Development of oral hygiene self-efficacy and outcome expectancy questionnaires. Community Dent Oral Epidemiol, 1997 Oct 1; 25(5): 337-42.
4. Macgregor ID, Balding JW, Regis D. Flossing behaviour in English adolescents. J Clin Periodontol, 1998 Apr 1; 25(4): 291-6.
5. Kornman KS. Antimicrobial agents. Dental plaque control measures and oral hygiene practices, 1986; 121-42.
6. Addy M, Moran J, Wade W. Chemical plaque control in the prevention of gingivitis and periodontitis. InLang NP, Karring T. Proceedings of the First European Workshop on Periodontology. London: Quintessence Publishing, 1994; 244-57.
7. Loe H. Chlorhexidine in the prevention and treatment of gingivitis. J Periodont Res., 1986; 21: 16-18.
8. Bonesvoll P, Gjermo P. A comparison between chlorhexidine and some quaternary ammonium compounds with regard to retention, salivary concentration and plaque-inhibiting effect in the human mouth after mouth rinses. Archives of Oral Biology, 1978 Jan 1; 23(4): 289-94.
9. Paraskevas S. Randomized controlled clinical trials on agents used for chemical plaque control. Int J Dent Hyg. 2005 Nov 1; 3(4): 162-78.
10. Jones CG. Chlorhexidine: is it still the gold standard?. Periodontol, 2000. 1997 Oct 1; 15(1): 55-62.
11. Fardai O, Turnbull RS. A review of the literature on use of chlorhexidine in dentistry. J Am Dent Assoc, 1986 Jun 1; 112(6): 863-9..
12. Rebstein F, Matter J, de Crousaz P, Jaccard F, Cimasoni G. Plak-out and Broxojet 3007. Clinical study. Schweiz Monatsschr Zahnmed, 1978 Oct; 88(10): 1155-65.
13. Bethesda M. A closer look at Ayurvedic medicine. Focus on complementary and alternative medicine. National Center for Complementary and Alternative Medicine (NCCAM). US National Institutes of Health (NIH), 2006; 12: 123-39.
14. Moran JM. Chemical plaque control–prevention for the masses. Periodontol 2000. 1997 Oct 1; 15(1): 109-17.
15. Lang N, Brecx MC. Chlorhexidine digluconate–an agent for chemical plaque control and prevention of gingival inflammation. J Periodont Res.1986 Nov 1; 21(s16): 74-89.
16. Addy M, Moran JM. Clinical indications for the use of chemical adjuncts to plaque control: chlorhexidine formulations. Periodontol 2000, 1997 Oct 1; 15(1): 52-4.
17. Shetty S, Pillai S, Sridharan S, Satyanarayana A, Rahul A. Comparative Efficacy of Chlorhexidine and a Herbal Mouth Rinse in Patients with Gingival Inflammation - A Clinical & Microbiologic Study, Asian Journal of Pharmaceutical Technology & Innovation, 01 (03); 2013.
18. Turesky S, Gilmore ND, Glickman I: Reduced plaque formation by the chloromethyl analogue of vitamin C. J Periodontol, 1970; 41: 41-43.
19. Hebbar A, Keluskar V, Shetti A. Oil pulling–Unraveling the path to mystic cure. J Int Oral Health, 2010 Dec 1; 2(4): 11-5.
20. Shetty S, Pillai S, Sridharan S, Satyanarayana A, Rahul A. Comparative efficacy of chlorhexidine and a herbal mouth rinse in patients with gingival inflammation-a clinical & microbiologic study. Asian Journal of Pharmaceutical Technology & Innovation, 2013; 1: 1-8.

21. Narayan A, Mendon C. Comparing the effect of different mouthrinses on de novo plaque formation. J Contemp Dent Pract, 2012 Jul 1; 13(4): 460-3.
22. Singh A, Purohit B. Tooth brushing, oil pulling and tissue regeneration: A review of holistic approaches to oral health. J Ayurveda Integr Med., 2011 Apr; 2(2): 64.
23. Bender JS, Thang h, Glogauer, Novel rinse assay for the quantification of oral neutrophils and the monitoring of chronic periodontal disease. J Periodont Res., 2006; 41: 214-220.