



EVALUATION OF EFFICACY OF TWO HERBAL MOUTHWASHES WITH 0.2% CHLORHEXIDINE: A DOUBLE BLIND RANDOMIZED CONTROLLED TRIAL.

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ABSTRACT

AIM: To evaluate the efficacy of natural herbal and commercially available herbal mouthwashes with 0.2% chlorhexidine in 12 to 15-year old school children.

MATERIAL AND METHODS: A Double-blind randomized controlled trial was conducted among 80 residential school children of age group 12 to 15 years who were selected based on inclusion and exclusion criteria and the selected sample was randomly divided into four groups with 20 subjects in each group. Plaque accumulation was assessed by Plaque Index (Silness and Loe 1964) and gingivitis by Gingival Index (Loe and Silness 1963). Baseline data was collected prior to start of study and after intervention, data was collected on 14th and 21st day.

RESULTS: The results of the present study showed that even though there is significant reduction of mean PI and GI between 4 groups, there is no statistically significant difference of Mean PI ($P=0.449$) and GI ($P=0.891$) score on 21st day among CHX and Hiora and CHX & Aloe vera in mean GI score ($p=0.442$).

CONCLUSION: Herbal mouthwashes can be used as an alternative to CHX in inhibition of plaque and gingivitis.

KEYWORDS : Chlorhexidine, Efficacy, Gingivitis, Herbal Mouthwash, Plaque.

INTRODUCTION:

Oral health is an integral part of general health and essential for well-being of human body. The interrelationship between oral and general health is proven by evidence.¹ To maintain good oral health, one needs to have good oral hygiene. Oral hygiene is the practice of keeping oral cavity healthy by brushing and flossing to prevent tooth decay and gum diseases. Good oral hygiene helps to prevent the build-up of plaque.²

Pellicle is a layer of organic material on all surfaces of oral cavity including hard and soft tissues and is the first stage of plaque formation.³ The micro-organisms present in oral environment produce dental plaque, consisting an average of more than 400 species in each gram of plaque.⁴ Plaque is the major etiological factor in gingivitis and periodontitis.⁵ Periodontal diseases are among the most common infectious diseases affecting oral cavity and can lead to destruction of the periodontal ligament, cementum, gingiva and alveolar bone.⁵ The bacteria in the plaque release toxins which cause swelling, redness and bleeding gums.⁶ Thus, plaque control holds the key to halt the progression of periodontal disease.⁵ Even though mechanical plaque control methods such as brushing and dental floss are the most indicated, economical and easy access for plaque control, they are not completely adequate for plaque control. So in addition to mechanical plaque control methods, chemical agents like mouthwashes, toothpaste, irrigators, chewing gums and varnishes play an important role.⁷ However, Mouthwashes are simple and widely accepted method to deliver anti-microbial agent which can be used as oral hygiene aids.⁷ They have the ability to deliver therapeutic ingredients and benefits to all inaccessible and interproximal surfaces.⁸ They have been used for centuries for medicinal and cosmetic purposes, but in recent years

the rationale behind use of chemical ingredients has been subjected to scientific research and clinical trials.⁸

Chlorhexidine (CHX) mouthwash is cationic bis-biguanide member of broad spectrum antibiotics⁷ with bacteriostatic ability at low concentrations and bactericidal at high concentrations with a substantivity of 12 hours.⁹ The mechanism of action of CHX is by rupturing of bacterial cell membrane resulting in cell death and inhibiting pellicle formation and plaque colonization.² Even though Chlorhexidine has been accepted as a gold standard anti plaque agent, its adverse-effects limit the long-term usage that includes taste alteration, paraesthesia, excess formation of supra gingival calculus, oral mucosal lesions in young patients, allergic responses, and staining of teeth and soft tissues.^{7,8,9}

So to overcome the disadvantages of conventional drugs, WHO advised researchers to investigate the possible use of plant extracts.⁹ Recently, herbal mouthwashes are gaining popularity as they contain naturally occurring ingredients called as Phytochemicals that achieve the desired antimicrobial and anti-inflammatory effects. These formulations may be more appealing because they work without alcohol, artificial preservatives, flavours or colors.^{5,10} Hence, purpose of present study was to evaluate the efficacy of natural herbal (Aloe vera) and commercially available herbal mouthwashes (Hiora) with 0.2% chlorhexidine in 12-15 year old school children.

MATERIALS AND METHODS:

The present study is a double-blind, parallel, randomized controlled trial employed to evaluate the efficacy of natural herbal and commercially available herbal mouthwashes with 0.2%

chlorhexidine among 12-15 year old school children. The study was conducted among inmates of a residential school located at Rajanaragam, East Godavari District, Andhra Pradesh. Prior ethical clearance was obtained from Institutional Ethical Committee (IEC) of Lenora Institute of Dental Sciences (LIDS) (File No:101/IEC/LIDS/2017). After explaining purpose, risks and benefits of study, permission was obtained from Principal of the school and Informed consent of all participants was obtained before start of the study.

Participants having DMFT score ≤ 3 , Plaque & Gingival scores ≥ 1 with previous history of not using mouth wash for last one month were included in the study. The participating subjects were free from systemic diseases with a minimum of 20 teeth in their oral cavity. Subjects undergoing orthodontic treatment, non-co-operative, physically challenged and with history of antibiotic therapy in last one month were excluded from the study.

The study was conducted during the month of August 2017 for a period of 21 days. Total sample of 80 participants following eligibility criteria were included in the study. Demographic information and oral examination were done to record baseline scores of gingival index (GI) ⁽¹¹⁾ and plaque index (PI) ⁽¹²⁾ according to Loe & Silness (1963) and Silness & Loe (1964) criteria respectively to assess gingival inflammation and plaque accumulation. Total sample (N=80) was randomly assigned into four groups (each group N=20) where group A received Chlorhexidine, group B received Hiora, group C received Aloe vera and group D received Placebo.

PROCEDURE:

Chlorhexidine (Saitech Medicare Pvt Ltd, Trilokpur Road, Kala Amb, Himachal Pradesh) and Hiora (The Himalaya Drug Company 4th Main, Peenya Industrial Area, Bangalore) mouthwashes are commercially available in the market that do not require any special preparation.

Incase of aloe vera, two parts of commercially available 98% pure Aloe vera juice (Patanjali Ayurved Ltd, Patanjali Food & Herbal park, Vill- Padartha, Laksar Road, Haridwar, Uttarkhand) was diluted with one part of distilled water 2:1. Commercially available distilled water was considered for Placebo group. Solutions were made of identical colour and taste to eliminate bias. Therefore, colouring and flavouring agents were added for all solutions to make it resemble as chlorhexidine mouth rinse. The contents of the solutions were known to the individual (other than principal investigator) who prepared the solution and were disclosed to the principal investigator at the end of the study. Each subject was given one of the test products with a given code according to assigned group. 10ml of mouth rinse using a measuring cup was dispensed for each individual and the subjects were instructed to swish mouthwash for 60 secs and then expectorate.

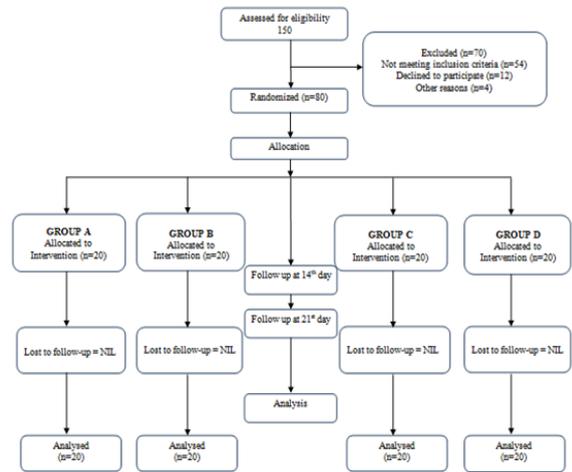
The procedure was performed once in morning after breakfast and was supervised by the examiner by visiting the school every day in morning and asked to repeat the same at night before retiring to bed. During intervention period, no influence on personal oral

hygiene procedures was exerted. The subjects were encouraged to maintain routine oral hygiene measures and also instructed to maintain strict compliance.

Participants were re-examined on 14th & 21st day of the study and oral examination was done to record gingival and plaque scores by the principal investigator following same criteria & methods to maintain uniformity. Single examiner was responsible for conducting the enquiry on adverse events and also monitoring of compliance. During study period no dropouts and withdrawals were encountered.

Statistical analysis:

Data collected during study was entered into excel sheet and subjected to analysis. Statistical analysis was done using SPSS software (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp). The analysis included descriptive statistics, paired t test for intragroup comparison, ANOVA & post hoc test for inter group comparisons. The statistical significance level was fixed at 0.05.



RESULTS

A Total 80 subjects included in the study were divided into four groups Group A (CHX), Group B (Hiora), Group C (Aloe Vera), Group D (Placebo)

Table 1: GENDER DISTRIBUTION OF PARTICIPANTS

Group	CHX	Hiora	Aloe vera	Placebo
Males	10	10	10	10
Females	10	10	10	10

CHX: Chlorhexidine

Table 1 shows gender distribution of study participants with 10 males and 10 females in each group i.e., CHX, Hiora, Aloe vera and Placebo respectively.

TABLE 2: COMPARISON OF MEAN PLAQUE SCORES BETWEEN DIFFERENT GROUPS.

	CHX	Hiora	Aloe vera	Placebo	Statistical inference (between different groups)
Baseline	1.04±0.27	0.90± 0.49	0.95±0.49	1.09±0.35	F=0.837 *P= 0.478
14 th day	0.75± 0.25	0.81± 0.33	0.93± 0.36	0.95± 0.30	F = 1.813 *P= 0.152
21 st day	0.52± 0.31	0.67± 0.38	0.81± 0.38	0.94± 0.18	F= 6.029 *P= 0.001 #Post Hoc: CHX vs Hiora= 0.449 (NS) CHX vs Aloe vera=0.03 CHX vs Placebo=0.001

## Statistical inference (within groups)	BL to 14 th day: df=19 t value= 11.34 P- Value= 0.001	BL to 14 th day: df=19 t value=1.58 P- Value=0.13	BL to 14 th day: df=19 t value=0.27 P- Value=0.79	BL to 14 th day: df=19 t value=3.51 P- Value=0.002	
	BL to 21 days= df=19 t value= 11.51 P Value=0.001	BL to 21 days= df=19 t value=2.09 P Value=0.05	BL to 21 days= df=19 t value=1.21 P Value=0.24	BL to 21 days= df=19 t value=2.49 P Value=0.022	
	14 th to 21 st day: df =19 t value=7.44 p value = 0.001	14 th to 21 st day: df =19 t value=1.92 p value = 0.07	14 th to 21 st day: df =19 t value=1.56 p value = 0.13	14 th to 21 st day: df =19 t value=0.23 p value = 0.81	

paired t test NS- Non significance

Hiora group (P-Value= 0.449).

*ANOVA- Analysis of variance #Post hoc test

Among CHX group, there was a statistically significant reduction in mean plaque scores from base line to 14th and 21st day. (P-Value= 0.001). In Hiora group statistically significant reduction in mean plaque scores was found only between baseline to 21st day (P-Value=0.05).

The comparison of mean plaque scores between different groups were mentioned in **table 2** and it was found that there was a statistically significant difference between mean plaque scores at 21 days (P-Value = 0.001). Pairwise comparison analysis by post-hoc test shows that statistically significant difference was found between CHX & aloe vera group (P Value =0.03) and also between CHX & placebo.(P-Value=0.001). However there was no statistical significant difference in mean plaque scores between CHX and

In placebo group statistically significant reduction in mean plaque scores was found between baseline to 14th day (P-Value=0.002) and baseline to 21st day (P-Value=0.02).

TABLE 3: COMPARISON OF MEAN GINGIVAL SCORES BETWEEN DIFFERENT GROUPS

	CHX	Hiora	Aloe vera	Placebo	Statistical inference (between different groups)
Baseline	1.13± 0.28	1.02±0.33	1.12±0.28	1.03±0.27	F= 0.746 *P= 0.528
14 th day	0.92±0.20	0.90±0.28	0.95±0.33	0.84±0.15	F=0.606 *P= 0.613
21st day	0.70±0.32	0.64±0.30	0.83±0.29	0.89±0.15	F=3.49 *P=0.01 #Post Hoc: CHX vs Hiora= 0.891 (NS) CHX vs Aloe vera= 0.442 (NS) CHX vs Placebo= 0.04
##Statistical inference (within groups)	BL to 14 th day: df=19 t value= 3.81 P- Value= 0.001 BL to 21 days: df=19 t value=4.71 P Value=0.001 14 th to 21 st day: df =19 t value=3.37 p value = 0.003	BL to 14 th day: df=19 t value= 1.86 P- Value= 0.07 BL to 21 days: df=19 t value= 4.03 P Value=0.001 14 th to 21 st day: df =19 t value=4.32 p value = 0.00	BL to 14 th day: df=19 t value= 2.62 P- Value= 0.01 BL to 21 days: df=19 t value= 3.50 P Value=0.002 14 th to 21 st day: df =19 t value=1.89 p value = 0.07	BL to 14 th day: df=19 t value= 3.24 P- Value= 0.004 BL to 21 days: df=19 t value= 2.59 P Value=0.018 14 th to 21 st day: df =19 t value=1.12 p value = 0.27	

*ANOVA- Analysis of variance #Post hoc test

paired t test NS- Non significance

gingival scores was found between baseline to 21st day (P=0.001) and 14th to 21st day (P-Value=0.001). Among Aloe vera group, there is a statistically significant reduction in mean gingival scores from base line to 14th (P-Value=0.01) and from base line to 21st day. (P-Value=0.002).

The comparison of mean plaque scores between different groups was mentioned in **table 3** and it was found that there was statistically significant difference between mean plaque scores at 21 days (P-Value = 0.001). Pairwise comparison analysis by post-hoc test shows that statistically significant difference was found between CHX & placebo.(P-Value=0.001). However there was no statistical significant difference in mean plaque scores between CHX & Hiora group (P-Value= 0.891) and CHX & Aloe vera (P-Value=0.44)

In placebo group statistically significant reduction in mean plaque scores was found between baseline to 14th day (P-Value=0.004) and baseline to 21st day (P-Value=0.01).

DISCUSSION

Chlorhexidine remains gold standard antiplaque and anti-gingivitis agent and its efficacy was evident. According to Kaur RK et al. herbal mouthwashes have advantages such as decreased side effects, more economical when compared to chlorhexidine.⁶ So the results of present study give an opportunity to determine the efficacy of

Among CHX group, there was a statistically significant reduction in mean gingival scores from base line to 14th and 21st day. (P-Value= 0.001). In Hiora group statistically significant reduction in mean

two herbal mouthwashes versus 0.2% chlorhexidine mouthwash on gingival status and plaque accumulations.

In present study, there is statistically significant difference (P -Value=0.001) in mean PI scores between 4 groups at 21 days. However there is no statistically significant difference in mean PI scores between CHX and Hiora. The results of present study are similar to the studies conducted by Nagesh Bhat et.al (2013)¹³ Shreya Shetty et.al (2013)¹⁴ Contrary to this, studies conducted by Rahul Gupta et al (2017)¹⁵, Ravi Varma Prasad KA et.al (2015)¹⁶ showed that Chlorhexidine had significant reduction in mean plaque scores when compared to its herbal alternative (Hiora).

In present study significant difference in mean plaque scores was observed between CHX & ALV ($P=0.03$) which is in accordance to study conducted by Chandrahas et.al¹⁷ and there was statistically significant reduction in plaque accumulation in Hiora when compared to placebo. This result was in accordance with the studies conducted by Sofrata et.al (2007) and Fouad Hussein Al-Bayat et.al (2016)⁶.

Similarly there is statistically significant difference (P - Value =0.001) in mean GI scores between 4 groups at 21 days. But there was no statistically significant difference in mean GI scores between CHX & Hiora. The results of present study are in similarity with the studies conducted by Ravi Varma Prasad KA et al (2015)¹⁶, Nagesh Bhat et.al (2013)³ and Shreya Shetty et.al (2013)¹⁴. In studies conducted by Kaur RK et.al (2014),⁶ Chandrahas et.al (2012)¹⁷ the results showed that the reduction in mean gingival scores was more in aloe vera when compared to placebo which is similar to the results in present study. This reduction might be because of anti-inflammatory effect of Aloe vera.¹⁸

In the present even though reduction in mean gingival scores was more in chlorhexidine group (Mean difference= 0.43) compared to aloe vera group (Mean difference= 0.28) from base line to 21 days, there was no statistically significant difference between chlorhexidine group and aloe vera group at 21 days ($P = 0.442$) which is in accordance with study conducted by Chandrahas et.al¹⁷($P=0.87$).

Within CHX group there is a statistically significant reduction in mean gingival and mean plaque scores. These results correlate with studies done by Devaki B et al (2015), Southern et.al (2000) and Malhotra et.al (2011).⁹ The reduction in PI and GI scores on using CHX could be attributed to prolonged antibacterial activity and dual antibacterial effect of CHX at tooth surfaces as a result of dicationic nature.¹⁹

Within hiora group there was statistically significant reduction in mean gingival scores ($P=0.05$) and mean plaque scores ($P=0.001$) between baseline & 21st day. This may be due to certain ingredients like persicain hiora which has anti-inflammatory and anti-fungal therapeutic effects.^{20,9}

Within aloe vera group there was statistically significant reduction in mean gingival scores ($P=0.002$) between baseline and 21st which was in accordance with studies conducted by Hegggers and Robson (1983), kaur RK et.al⁶ and Ajmer et.al¹⁸ and there was no significant difference in mean plaque scores at end of 21st day which was contrary to study conducted by kaur RK et.al.⁶ The decrease mean gingival and plaque scores may be due to barbolin and aloe emodin in aloe vera which has numerous anti-inflammatory agents like carboxypeptidase which inactivates bradykinin thereby inhibiting prostaglandin synthesis and oxidation of arachidonic acid which might decrease inflammation and relieves pain.^{6,17} Vazquez et.al stated Aloe vera decreases oedema and also prevents migration of poly morpho nuclear leucocytes. Barrantes and Guinea et.al stated that Aloe vera inhibits the stimulated granulocyte matrix metallo proteinases inhibiting cyclo-oxygenase and lipo-oxygenase pathways.⁶ The antimicrobial effect of ALV has been demonstrated in an in vitro study where it was reported to inhibit the growth of

diverse oral microorganisms such as Streptococcus mutans, Streptococcus sanguis, Actinomyces viscosus and Candida albicans.¹⁰

Variations in plaque and gingival scores may also have been influenced by Hawthorne effect or the tendency of participants to improve behaviour because of the expectation created by the situation.¹⁹

Even though the results demonstrated relevant differences in the anti-microbial effect of herbal mouthrinse, there is a need of other invitro and invivo investigations to confirm the results presented in this study. Further research need to be conducted on larger sample size and long term studies to determine the substantivity and anti-microbial effect on gingivitis and plaque accumulation.

CONCLUSION

The present study concludes that there was reduction in mean plaque and gingival scores among test groups along with chlorhexidine and the difference was statistically significant in mean plaque scores. Herbal products considering being safer with use of no chemical additives, there is an increasing communal desire to gravitate on naturally occurring compounds. So, herbal mouthwashes can be used as an anti-plaque and anti-gingivitis agents and can be preferred over chlorhexidine as they are cost effective and has limited side-effects.

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