ANTI PLAQUE AND ANTI GINGIVAL EFFECT OF CHLORHEXIDINE (0.2%) AND AQUEOUS STEVIA MOUTH RINSES - AN INVIVO CLINICAL TRIAL

Type of manuscript: Original study
Running Title: The Anti-plaque and Anti-gingival effectiveness of Stevia mouthrinse

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Abstract:
Background: The main enemy for oral diseases is dental plaque. Numerous oral hygiene measures have been adapted to eliminate dental plaque, one among them will be the use of chlorhexidine mouthwash which is effective against wide array of micro-organism. But the major adverse effect of chlorhexidine mouthwash will be staining and alteration of taste. Inorder to overcome the adverse effect, various naturally occurring antimicrobial agents have been incorporated such as aqueous stevia mouthwash to prevent the formation of dental plaque.
Aim: To compare the anti-plaque and anti-gingival effectiveness of Aqueous Stevia and chlorhexidine containing mouthwash.
Materials and Methods: A randomized controlled trial was conducted among 20 healthy individuals. The participants were randomly allocated into two groups: Group I(Experimental group-Stevia), GroupII (Controlled group - Chlorexidine). Before conducting the trial, the study design was explained to the qualifying participant and informed consent was obtained from the voluntary patients who were willing to participate in the study. Erythrosin disclosing solution was used to disclose the plaque. The plaque index and gingival index was recorded at baseline and 7th day. Wilcoxon signed Rank test used to compare the mean differences before and after treatment within each group for Plaque and Gingival index score.
Results: A statistically significant reduction in mean Plaque and Gingival Index score was observed in both Group I and Group II at baseline and 7th day follow up. But there was no statistically significant difference was observed between the groups at 7th day of follow up.

Conclusion: To conclude, there was a statistically significant reduction was observed in mean plaque index and gingival index score of Group I and Group II at 7th day of follow up. Hence, Stevia mouthwash can be used as an effective antimicrobial oral hygiene measure against plaque and gingival diseases.

Keywords: Plaque, Mouthwash, Oral hygeine, Index, Gingivitis

INTRODUCTION:

Dental plaque is the community of microorganisms found on a tooth surface as a biofilm, embedded in a matrix of polymers of host and bacterial origin [1,2]. It is a bacterial biofilm, and is made up of a wide variety of over 400 species of bacteria growing on the hard and soft tissues of the mouth and embedded in an extracellular matrix (polysaccharides, EPS) of bacterial and salivary origin. [3,4] Of clinical relevance is the fact that biofilms are less susceptible to antimicrobial agents, while microbial communities can display enhanced pathogenicity (pathogenic synergism) [5]. The structure of the plaque biofilm might restrict the penetration of antimicrobial agents, while bacteria growing on a surface grow slowly and display a novel phenotype, one consequence of which is a reduced sensitivity to inhibitors [6]. Plaque is natural and contributes (like the resident microflora of all other sites in the body) to the normal development of the physiology and defenses of the host [7].
Only a relatively small proportion of the bacteria present in the plaque are implicated in disease states such as caries and periodontal disease [8,9]. However, with regard to oral health and disease, it is important to determine plaque biofilm architecture as this will have profound effects both on the removal of potentially harmful bacterial metabolites from the biofilm and also on the delivery of substrates and therapeutics to the deeper layers of the plaque closer to the oral tissues.

The principle means of preventing the progression of dental plaque is through mechanical removal by tooth brushing(10).But for the people who are mentally and physically challenged and as well as those people who lack that skill and motivation, mouthwashes aids in preventing the plaque accumulation(11).Its the duty of dental professionals to recommend effective oral hygiene to control dental plaque and accumulated inflammatory components to maintain optimal oral health(12).The mouthwash has various therapeutic uses which includes reducing inflammation, halitosis and deliver fluoride for caries prevention(13).However mouthwash should only be used as conjunction with mechanical measures(14).

Chlorhexidine mouthwash is found to possess the most superior anti-plaque property and is considered to be the gold standard. It has been initially produced as anti-viral agent. But its use began as a disinfectant which has a wide anti-microbial spectrum. But there is existence of some side effects which include brownish discolouration of teeth and long term use of this mouthwash may impair taste sensation(15,16).Thus many alternative herbal mouthwashes has been introduced on daily basis to maintain the oral hygiene and to treat plaque induced gingival diseases as effectively as chlorhexidine(17).One among them is aqueous Stevia mouthwash.

It is a natural sweetener and a perennial shrub of the Asteraceae family, native of Paraguay and Brazil. The glycosides Stevioside and Rebaudioside A are present in the S. rebaudiana leaves and they taste approximately 200 and 300 times sweeter than sucrose. Stevia rebaudiana sweeteners have also shown to be non-cariogenic [18] It also has anti-plaque and anti-gingival activity. Thus this study is designed to evaluate and compare the anti-plaque and anti-gingival effectiveness of chlorhexidine and aqueous stevia mouthwash.

Materials and Methods:

Study Design:

Invivo clinical trial.

Study Population:

Final year dental students of saveetha dental college and hospital, chennai were recruited for the clinical trial.

Eligibility Criteria:

Inclusion criteria

a) Apparently Healthy patients without any known history of systemic illness above 18- 25yrs of age.
b) Dental students with Good to Fair Plaque index score.
c) Dental students with mild to moderate gingivitis.
d) Dental students with habit of tooth brushing twice daily

Exclusion criteria

a) Dental students with positive history of usage of anti microbial therapy and routine use of oral antiseptics in the previous 3 months
b) Dental students with history of allergic and idiosyncratic reactions to product ingredients.
c) Dental students undergoing orthodontic treatment.
d) Dental students with presence of systemic diseases that could alter the production or composition of plaque and saliva.

Sample Size Determination

Sample size was calculated by using a priori by G*Power 3.1.2software. The minimum sample size of each group was calculated, following these input conditions: power of 0.95 and $P \leq 0.05$ and sample size arrived were 10 per group.

Ethical clearance:

Prior to the start of the study, ethical clearance was obtained from the institutional ethics committee, Saveetha university

Armamentarium:

The following equipment/instrument/materials were used for the study:

- Dental chair
- Dental operating tool
- Tray sheet
- Disposable head cap
- Disposable Mouth mask
- Disposable gloves- small size
- Disposable cups
- Disinfecting solution
- Kidney tray
- Plane mouth mirror
- Tweezer
- Proforma

Intervention Groups (Refer Table 1): The participants were randomly allocated to Group I and Group II.

Group I- Stevia Mouthwash

Group II- Chlorhexidine mouth wash

Method of Preparation of Stevia mouth wash-

According to the Standard textbook of Pharmaceutics, Indian Pharmacopia 2007, 5th edition [19,20] For 1% aqueous suspension of Stevia , 1 gram of Stevia powder was dissolved in a 2% hydroxypropyl methylcellulose (suspending agent)

dispersed with constant stirring for 2 hours, 0.5 ml of glycerin was added and the volume of 100ml was made up with distilled water.

Study Procedure:

Step 1: Obtaining preoperative details and informed consent from study participants-Prior to the treatment, a careful medical and dental history was taken. Preoperative data for each participant was recorded in the predesigned proforma which includes age, gender and address. The study design was explained to the qualifying participant and informed consent was obtained from the voluntary patients who were willing to participate in the study.

Step 2: Application of disclosing solution- Disclosing solution was generously applied to the surfaces of the teeth with the help of applicator brush. The study participants were instructed to rinse the mouth (Figure 1).

Step 3: Scores and Criteria for recording Plaque (Silness and Loe) and Gingival index (Loe and Silness) (Refer Table 2 and 3)-Plaque index was recorded for the indexed teeth.
Gingival index was recorded for the entire dentition and in all the surfaces of the teeth prior to oral prophylaxis. The surfaces include
• Distobuccal,
• Midbuccal,
• Mesiobuccal
• Palatal surface.
The mean score of plaque and gingival index is recorded in the pre structured proforma

Step 4: Oral hygiene instructions and tooth brushing technique-
A standardized toothbrush and the toothpastes were allocated according to the group. Oral hygiene instructions withan emphasize on the appropriate brushing technique were given.

The following Instructions were given to all study subjects:
• To use the given soft bristled tooth brush and anti-cavity tooth paste .
• To take a pea sized tooth paste or ½ of the length of the head of the brush sized paste should be taken.
• To brush teeth for a minimum of 2 minutes twice a day

Brushing techniques:
• To place the bristles at a 45-degree angle to the teeth. Slide the tips of brush under the gums.
• To jiggle the bristles very gently so that any plaque growing under the gum will be removed.
• To be sure to brush the outside, the tongue side and the chewing surfaces of your teeth.
• For the front teeth, to brush the inside surfaces of the upper and lower jaws by tilting the brush and making several up and down strokes.

Direction of use : Fill cap to the "fill line" (15 mL). Swish in mouth undiluted for 30 seconds, then spit out. Use after breakfast and before bedtime. Or, use as prescribed. **NOTE**: To minimize medicinal taste, do not rinse with water immediately after use.

Step 5: Follow up at 7th day- The above mentioned steps were repeated at 7th day of follow up.

Outcome Measure

The investigator recorded the mean score of plaque and gingival index after the use of tested products at baseline and 7th day
Statistical Analysis.
• Data was entered in Microsoft excel spread sheet and analysed using SPSS software (version 17).
• Numerical data were presented as mean and standard deviation values.
• For test, a p value of <0.05 is to be considered statistically significant.
• **Wilcoxon signed Rank test** used to compare the mean differences before and after treatment within each group for Plaque and Gingival index score.

RESULTS:
Figure 2 depicts changing trends of Mean Plaque Index score of Group I, II at baseline and 7th day. A significant reduction was observed in mean plaque Index score of Group I from baseline value of 0.524 to 0.49 at 7th day of follow up and in Group II, Plaque Index score dropped from 0.49 to 0.43. Figure 3 shows the Changing trends of Mean Gingival Index score of Group I, II at baseline and 7th day. A significant reduction was observed in mean Gingival Index score of Group I from baseline value of 0.55 to 0.46 at 7th day of follow up and in Group II, Gingival Index score dropped from 0.54 to 0.48 respectively. Table 4: Comparison of Mean Plaque and Gingival Index scores at Baseline, and 7th day time points of Group I and II. A statistically significant difference was observed in mean Plaque Index and Gingival Index score at Baseline and 7th day of follow up using Wilcoxon signed rank test.

Table 1: Products tested and its composition

GROUP	PRODUCTS	COMPOSITION*
I	Aqueous Stevia mouthwash	Stevia powder, Hydroxypropylmethyl cellulose, glycerol
II	Chlorhexidine mouthwash	Chlorhexidine gluconate solution I.P. dilute to chlorhexidine gluconate 0.12% W/V in pleasantly flavored aqueous base

*As provided by the manufacturer

Table 2: Scores and criteria for Plaque Index (Silness and Loe-1964)

Score	Criteria
0	No plaque
1	A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen only by running a probe across the tooth surface.
2	Moderate accumulation of soft deposits within the gingival pocket, on the gingival margin and/or adjacent tooth surface, which can be seen by naked eye.
3	Abundance of soft matter within the gingival pocket and/or on the gingival margin and adjacent tooth surface.

Table 3: Scores and criteria for Gingival Index (Loe and Silness -1963)

Score	Criteria
0	No inflammation.
1	Mildinflammation, slight change in color, slight edema, no bleeding on probing.
2	Moderate inflammation, moderate glazing, redness, bleeding on probing.
3	Severe inflammation, marked redness and hypertrophy, ulceration, tendency to spontaneous bleeding.

Table 4: Comparison of Mean Plaque and Gingival Index scores at Baseline, and 7th day time points of Group I and II

Index scores		Time periods	Mean	Std. Deviation	Std. Error Mean	P value
Plaque Index score	Group I	Baseline	0.54	0.12	0.04	<0.05
		7th day	0.46	0.12	0.04	
	Group II	Baseline	0.49	0.12	0.04	<0.05
		7th day	0.42	0.10	0.03	
Gingival Index score	Group I	Baseline	0.55	0.13	0.04	<0.05
		7th day	0.46	0.13	0.04	
	Group II	Baseline	0.54	0.12	0.03	<0.05
		7th day	0.48	0.11	0.03	

Wilcoxon signed Rank test(P<0.05)

Figure 1: Application of Disclosing agent

Figure 2: Changing trends of Mean Plaque Index score of Group I, II at baseline and 7th day

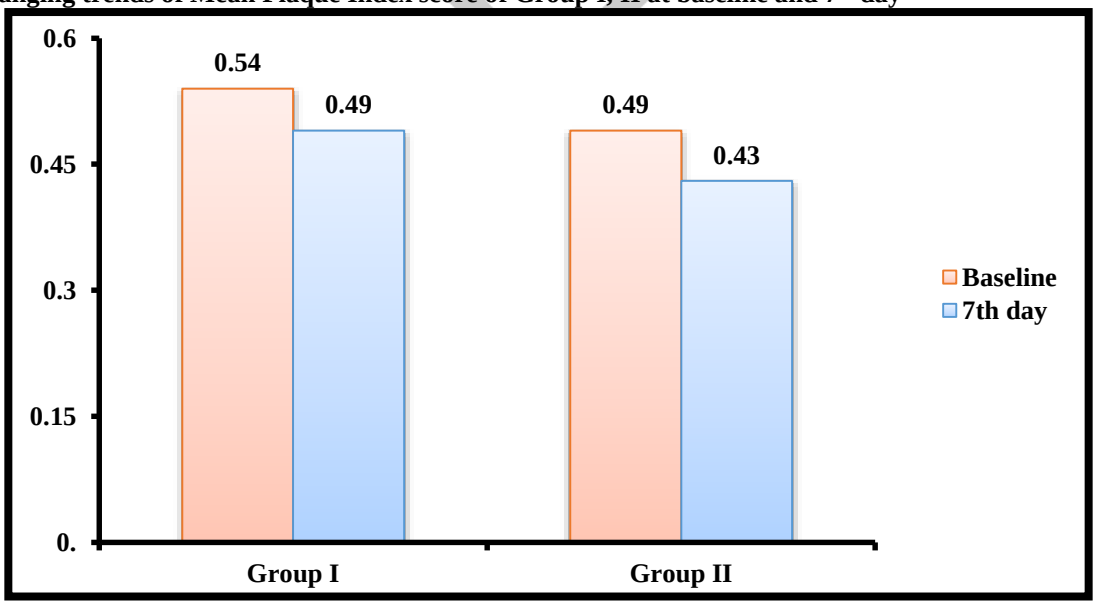
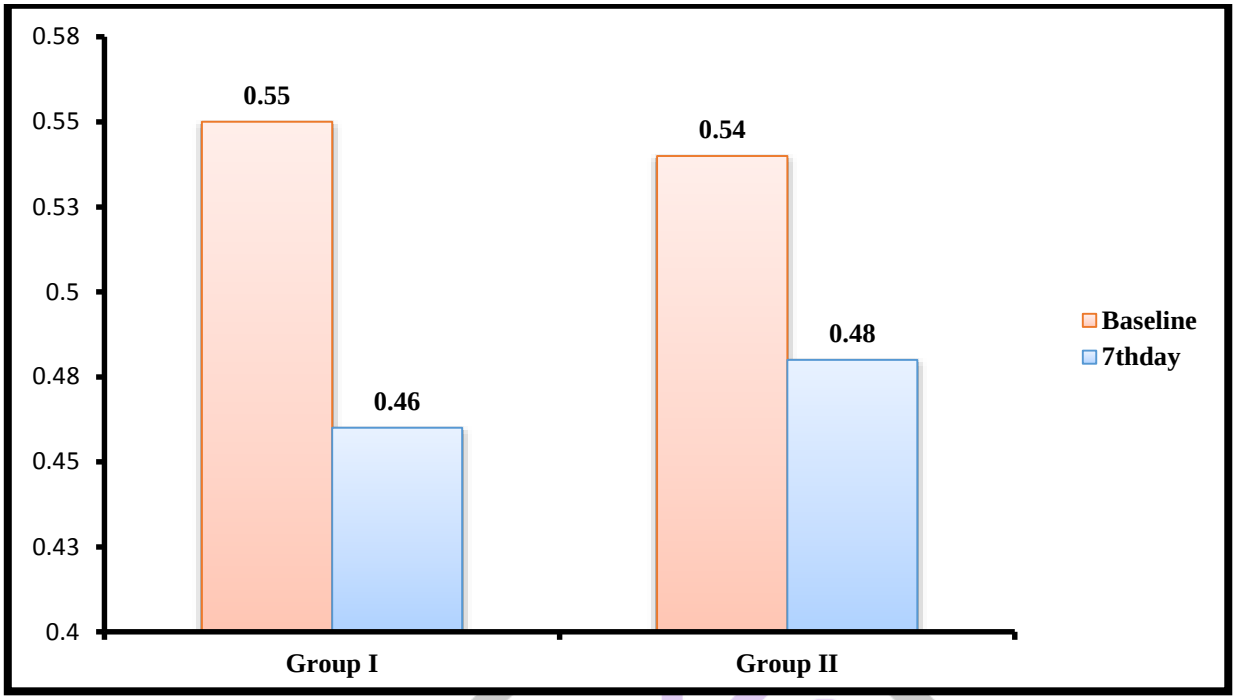


Figure 3: Changing trends of Mean Gingival Index score of Group I, II at baseline and 7th day.



DISCUSSION:

In the present study, a statistically significant reduction in mean plaque index and gingival index scores was observed in both the groups at baseline and 7th day using Wilcoxon signed Rank test. Eventhough there was no significant difference in mean plaque index and mean gingival index was observed between the groups at 7th day of follow up,but Group II showed an higher reduction when compared to Group I .Our results were found to be in contrast when compared with the study done by Vandana et al.,[21]where Stevia mouth rinse was found to be effective. It was found that there was a significant reduction in mean PlI and GI scores for chlorhexidine, sodium fluoride and stevia groups. However, there was increase in PlI and GI scores among the participants who used placebo rinse.

Chlorhexidine had significant improvement on plaque and gingival scores when compared to the other reference groups at 3 months evaluation as similar to studies reported by Löe and Schiott,[22] Flötra et al.,[23] Löe et al.,[24] Lang et al.,[25] Jenkins et al.[26]

This study showed that stevia mouthrinse is superior in reducing in mean PlI and GI scores at the end of 6 months as compared to chlorhexidine, sodium fluoride and placebo groups because Stevia contains tannins, xanthines (theobromine and caffeine) and flavonoids that have antiplaque activity as similar to studies reported by Menaker,[27] Yabu et al.[28] Moreover Stevia is heat-stable, resistant to acid hydrolysis, and nonfermentable by oral bacteria reported by Kinghorn et al.[29]

Interestingly in the study of Vandana et al., the initial lesions which were diagnosed according to ICDAS II criteria remained the same throughout the study period which signifies that stevia mouthrinses were effective in arresting the carious lesions at the initial stages.

Conclusion: To conclude, there was a statistically significant reduction was observed in mean plaque index and gingival index score of Group I and Group II at 7th day of follow up. Hence, Stevia mouthwash can be used as an effective antimicrobial oral hygiene measure against plaque and gingival diseases.

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