

ORIGINAL ARTICLE**PRELIMINARY ANTIPLAQUE EFFICACY OF ALOE VERA MOUTHWASH ON 4 DAY PLAQUE RE-GROWTH MODEL: RANDOMIZED CONTROL TRIAL****Gupta Rajendra Kumar¹, Gupta Devanand², Bhaskar Dara John², Yadav Ankit³, Obaid Khursheed⁴, Mishra Sumit⁵****ABSTRACT**

BACKGROUND: Due to increasing resistance to antibiotics and rising incidence of oral diseases, there is a need for alternative treatment modalities to combat oral diseases. The aim of the present study was to access the effect of Aloe vera mouthwash on the dental plaque in the experimental period of 4 days and to compare it with the bench mark control chlorhexidine and placebo (saline water).

MATERIAL AND METHODS: A total of 300 systemically healthy subjects were randomly allocated into 3 groups: Aloe vera mouthwash group (n=100), control group (=100)–chlorhexidine group and saline water-Placebo (n=100). To begin with, Gingival index (GI) and plaque index (PI) were recorded. Then, baseline plaque scores were brought to zero by professionally cleaning the teeth with scaling and polishing. After randomization of the participants into three groups they were refrained from regular mechanical oral hygiene measures. Subjects were asked to swish with respective mouthwash (Aloe vera mouthwash, 0.2% chlorhexidine gluconate mouthwash, or normal saline) as per therapeutic dose for 4 days.

RESULTS: The results showed that Aloe vera mouthrinse is equally effective in reducing plaque as Chlorhexidine compared to placebo over a period of 4 days. There was a significant reduction on plaque in Aloe vera and chlorhexidine groups and no statistically significant difference was observed among them ($p>0.05$). Aloe vera mouthwash showed no side effects.

CONCLUSION: The results of the present study indicated that Aloe vera may prove an effective mouthwash due to its ability in reducing dental plaque.

KEYWORDS: Mouthwash, plaque, chlorhexidine

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INTRODUCTION

Dental diseases are recognized as major public health problem throughout the world. Numerous epidemiological studies showed that diseases such as tooth decay and diseases of the periodontium are among the most common afflictions of mankind. Dental plaque plays a major role in the aetiology of periodontal disease. The mainstay of preventing periodontal disease is the control of

plaque and thus the prevention of plaque induced gingivitis (1).

Various synthetic chemical agents have been evaluated over the years with respect to their antimicrobial effect in oral cavity. The benchmark control in the removal of plaque is chlorhexidine. But, it cannot be used for a long duration because it has many side-effects like altered taste sensation and staining of tongue. Chemical plaque control agents are used as an adjuvant since they have the ability to inhibit growth and metabolism as well

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as colonization of bacteria; however, all are associated with various side effects (2-4). Thus, patients are going away of modern day medicines, and they prefer using herbal preparations which are efficient without causing any side effects. Use of herbs for dental care is very common in indigenous system of medicine and herbs like *Terminalia chebula*, *Aloe vera*, *Azadirachta indica*, piper betle, *Ocimum sanctum* possess antibacterial, ulcer healing, antiplaque and anti halitosis properties (5).

The present study includes *Aloe vera* extract which may be tested as one such oral hygiene aids to reduce plaque formation. The main active ingredients in the *Aloe vera* gel are aloin, aloemodin, aloemannan, acemannan, aloeride, naftoquinones, methylchromones, flavonoids, saponin, sterols, amino acids and vitamins. Vast literature has mentioned its use worldwide like in Egypt, South Africa, India, China, Mexico and Japan for various ailments like burns, hair loss, skin infections, haemorrhoids, sinusitis, and gastrointestinal (GI) pain. It is also a wound healer for bruises, X-ray burns, insect bites; and anti-helminthics, somatics, anti-arthritis. *Aloe vera* has been used for various skin conditions, including radiodermatitis, frostbite, psoriasis and genital herpes infection. Its pharmacological actions include anti-inflammatory, antibacterial, antioxidant, antiviral and antifungal actions (6-9).

Literature is abundant on the health beneficial effects of *Aloe vera* but to date, no studies have been conducted to test its antiplaque efficacy on 4 day plaque re-growth model. Hence, this study was conducted to evaluate the efficacy of *Aloe vera* on the clinical level of dental plaque and to compare it with benchmark antiplaque agent chlorhexidine. This study compared the “*de-novo*” plaque formation between the 3 groups (*Aloe vera* group, chlorhexidine group, and placebo group) during a 4 day cessation of mechanical plaque control using a 4 day plaque re-growth model (10).

MATERIAL AND METHODS

In this double blind randomized control trial; a three group's parallel study was conducted in the Department of Public Health Dentistry on voluntary undergraduate students of Teerthankar Mahaveer Dental College and Research Centre.

Protocol was approved by the Institutional Review Board (IRB) of Teerthankar Mahaveer University. All subjects signed an IRB approved consent form. The study was carried out according to the guidelines of the Declaration of Helsinki (11) for biomedical research involving human subjects.

Inclusion and exclusion criteria: Systemically healthy subjects with gingival index ≤ 1 (as a mean value for all tooth surfaces scored), a dentition of ≥ 20 teeth with a minimum of 5 teeth per quadrant and DMFT index ≥ 2 were enrolled in this study. The following subjects were excluded however: those using any mouthwash during the study, those having antibiotic therapy within the last 2 weeks, those having a history of hypersensitivity to any product used in the study, those with a recent tooth extraction and those on any anticoagulant medication.

Sample size and randomization: All the undergraduate students of Teerthankar Mahaveer Dental College and Research Centre were subjected to clinical examination, and a sampling frame ($n = 1410$) was prepared for those who fulfilled the inclusion and exclusion criteria. A sample size of 300 was arrived at by using Statistical Software. A total of 300 volunteers were randomly allocated into the three study groups through computer-generated random numbers. Random allocation of mouthrinses was done using the lottery method.

The total sample size was 300 (100 subjects in each group). The sample size was calculated for α error fixed at $< 5\%$ ($p < 0.05$) and β fixed at 20%, expected mean difference = 2.691 and standard deviation = 2.319. Based on the above calculation, the minimum sample required in each group was 100 subjects.

In Group 1 ($n = 100$) *Aloe vera* mouthwash was given to the participants and instructed to use 10ml twice a day for 60 seconds for the experimental period of 4 days; in Group 2 ($n = 100$) Chlorhexidine mouthwash was given to the participants and instructed to use 10ml twice a day for 4 days and in Group 3 ($n = 100$) saline water was given as the mouthwash.

With the help of the Department of Pharmacy, all the three solutions were made of same color. They were kept in coded containers and decoded later. All recordings were made by the same examiner. After disclosing plaque, baseline plaque scores were brought to zero by

professional scaling and polishing with rubber cups and an abrasive paste. Intraoral colored photograph were taken. Then subjects were assigned randomly to 1 of the 3 groups (of 100 subjects each) who were refrained from regular oral hygiene measures (tooth brushing and dental flossing), except for swishing with respective mouthwash as per therapeutic doses were taken. Students were instructed to rinse their mouths with 10ml of mouthwash twice daily after breakfast and others after lunch for 4 days for one minute and not to rinse with water thereafter. This regimen for each group was followed for 4 days, and participants were asked to enter in a diary provided to them (so that they do not forget to rinse). Plaque index scores were re-evaluated on the 4th day by the same examiner who was unaware of the mouthwash used by the subjects. A trained single examiner recorded the findings at both the intervals. Using kappa statistics, intra-examiner reliability was 0.86 for the plaque index. Side-effects of mouthwashes were evaluated from subjects of all the 3 groups by means of a questionnaire as well as through clinical examination. Subjects returned to their habitual oral hygiene measures after the completion of the study period.

Preparation of Aloe vera mouthwash: The 100% pure *Aloe vera* juice was provided by the Natura Biotechnol pvt Ltd, India. The placebo solution and the control were taste-matched, with identical astringency, consistency, and ingredients as much as possible by the *Aloe vera* research group of the Faculty of Pharmacy, Teerthankar Mahaveer University.

Plaque analysis: After 4 days the plaque was disclosed using disclosing solution and the scores were recorded at six sites per tooth using the Quigley and Hein plaque index modified by Turesky-Gilmore-Glickman (12).

Statistical analysis: The data were analyzed using SPSS version 21. ANOVA and post-hoc LSD were used for analysis. P value of 0.05 was taken to be significant.

RESULTS

Plaque score: The mean and range of plaque scores for Groups I, II, III are depicted in Table 1.

ANOVA (Table 2) was calculated to assess the intra- and inter-group variations for plaque. There was a significant decrease in the plaque in both the *Aloe vera* and chlorhexidine groups over a period of 4 days ($P < 0.05$) (Tables 2, 3). There was a progressive decrease in the plaque score at 5% level of significance in both chlorhexidine and *Aloe vera* groups. Chlorhexidine group showed maximum decrease as compared to *Aloe vera* group but the difference was not statistically significant. Multiple comparisons were obtained by Post-hoc LSD. The difference in the decreases in plaque ($P=1.921$ at the 4th day) between Group I and Group II were not statistically significant. However, the differences between *Aloe vera* and the placebo group and chlorhexidine and the placebo group were significant ($P < .05$). The study showed that there was no difference in the *Aloe vera* and chlorhexidine groups as far as plaque scores are concerned.

Opinions of the participants were evaluated after 4 days of mouthwash use. Staining (mild brown discoloration of teeth) was found in 70 subjects in the chlorhexidine group (estimated visually and from the colored photographs). Sixty-five subjects in chlorhexidine group reported an unpleasant taste (mild alteration in taste for salty foods/drinks). No such side effects were observed in *Aloe vera* group.

Table 1: Mean and range of plaque scores in different groups

Group	Mean	Minimum	Maximum	SD
Group 1		1.34	3.89	0.29
Group 2	3.10	1.25	3.78	0.25
Group 3	4.64	3.78	6.15	0.49

Table 2: Analysis of variance for plaque scores of different groups

Analyses of variance	Sum of Squares	Mean Square	F	Sig.
Between Groups	6.128	7.91	8.927	0.001
Within Groups	11.784	0.581		
Total	17.912			

Table 3: - Post – hoc LSD test for multiple comparisons

Variable	(I) Group	(J) Group	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Lower Bound
Plaque (4 days)	<i>Aloe vera</i>	Chlorhexidine Placebo	.17651 .17651	1.921 .001	.090 -246.18	77.15 112.87
	Chlorhexidine	<i>Aloe vera</i> Placebo	.17651 .17651	1.921 .001	-77.15 -187.98	-.090 -241.35
	Placebo	<i>Aloe vera</i> Chlorhexidine	.17651 .17651	.001 .001	-187.98 -241.35	246.18 187.98

(I) and (J) designations according to post-hoc analysis by SPSS
The mean difference is significant at the .05 level

DISCUSSION

There is an increase in the use of mechanical and chemical plaque control agents to prevent dental caries and periodontal disease. Some of the methods are proper and regular tooth-brushing, flossing and rinsing with mouthwashes. Various chemical mouthwashes are available in market but are associated with side-effects like immediate hypersensitivity reactions, toxicity, tooth staining, etc. Alternative medicines may be developed from medicinal plants as these plants contain natural phytochemicals, and hence, can replace synthetic drugs (13-15). *Aloe vera* is widely used in various ailments of the body but it is seldom tested as an antiplaque agent. Thus, this study was undertaken to know the antiplaque efficacy of *Aloe vera* on 4 day plaque re-growth model. The study is based on the guidelines of American Dental Association Council on Scientific Affairs for evaluating the clinical efficacy of chemotherapeutic mouthrinses (American Dental Association Council on Scientific Affairs, 1997).

Students were instructed to rinse their mouths with 10ml of solution for a period of 1min after breakfast and lunch, and then, they were told not to rinse their mouth with water or drink anything for half an hour (16). According to Van Strydonck et al (17), alcohol-free rinse was as effective as one containing alcohol in controlling plaque and hence, we used alcohol free chlorhexidine in our study.

Aloe vera is commercially used for many pharmaceutical and cosmetic purposes but literature regarding its anti plaque efficacy is scarcely available. *Aloe vera* at 100%

concentration was found to have maximum antibacterial effect *in vitro* study. Thus, in this study, *Aloe vera* at 100% concentration was used. In the study, we found no statistically significant difference between the antiplaque efficacy of chlorhexidine and that of *Aloe vera* mouthwashes over a period of 4 days. To our knowledge, the present study is the first randomized control trial assessing the antiplaque efficacy of *Aloe vera* on 4 days plaque re-growth model. The result of the studies conducted by Villalobos *et al.* (18) and Chandrahas *et al.* (19) differed from our study. This difference may be due to different study designs and different concentrations of mouthwashes used; however, the results of a recent clinical trial conducted by Karim *et al.* (20), which have used same concentration of mouthwash as in the present study, is comparable with this study.

Aloe vera has demonstrated antibacterial action against a range of bacteria particularly against *Streptococcus mutans*, which account for its anti-plaque action (21). Some of the constituents of *Aloe vera* like Vitamin C, hyaluronic acid and dermatan sulfate are involved in collagen synthesis, and hence, provide relief in swelling and bleeding gums (20).

The test group showed significant reductions on plaque at the end of the trial (*Aloe vera*, 60%; CLX, 61%). This is consistent with previous studies. Nevertheless, this difference was not significant, showing that *Aloe vera* had a potential similar to chlorhexidine as an anti-plaque agent. The effect of saline water on plaque status indicated that simple mechanical rinsing with saline water is not adequate to show any positive

results. Subjects on 0.2% chlorhexidine gluconate mouthwash exhibited mild brown staining of teeth, which was not observed in *Aloe vera* mouthwash subjects. Moreover, *Aloe vera* mouthwash is cost-effective as compared to 0.2% chlorhexidine gluconate mouthwash. In order to minimize the potential bias of novelty factor (participants are motivated by themselves to improve their oral hygiene) and lack of compliance among participants, they were asked to rinse everyday in front of the investigator.

Within the limitations of this 4-day “*de-novo*” plaque formation study, it was concluded that there was no statistical difference between 0.2% chlorhexidine gluconate mouthwash and *Aloe vera* mouthwash. Therefore, in low socio-economic status population, presently tested *Aloe vera* mouthwash can be a better alternative to 0.2% chlorhexidine gluconate mouthwash. Thus, when socio-economic factors, side effects of chlorhexidine and/or preference of the population for natural products need consideration, presently tested herbal mouthwash may be considered as a good alternative. Therefore, herb-based formulations are likely to replace *Chlorhexidine* as soon as intense antimicrobial, palatable, and cost-effective preventive strategies are available. Demand for alternative medicine is on increase (22, 23, 24). However, future studies (with a long-term rinsing period) can be done to extrapolate the advantages and disadvantages of this herbal product.

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