Effect of *Aloe vera* Mouthwash on Periodontal Health: Triple Blind Randomized Control Trial

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Abstract

Background: With the increasing incidence of periodontal diseases and development of antibiotic resistance, the global need for alternative treatment modalities, safe, effective, and economical products is the need of time. *Aloe vera* is a medicinal plant which has the greater medicinal value and enormous properties for curing and preventing oral diseases disease.

Aim: The aim of the study was to access the effect of *Aloe vera* mouthwash on the dental plaque and gingivitis and comparing it with the bench mark control chlorhexidine and placebo.

Material and methods: 345 healthy subjects were randomly allocated in 3 groups to the test group (n=115) – mouthwash containing *Aloe vera*, Control group (n=115) –chlorhexidene group, Distilled water - Placebo (n=115). Plaque Index (PI) and Gingival Index (GI) were assessed at days 0, 15 and 30. Subjects were asked to rinse their mouth with the stated mouthwash, twice a day, during a 30-day period.

Results: Our result showed that *Aloe vera* mouthrinse is equally effective in reducing periodontal indices as Chlorhexidine. The results demonstrated a significant reduction of gingival bleeding and plaque indices in both groups over a period of 15 and 30 days as compared to placebo group. There was a significant reduction on plaque and gingivitis 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 as seen with chlorhexidine.

Conclusion: The results of the present study indicate that *Aloe vera* may prove to be an effective mouthwash owing to its ability in reducing periodontal indices.

Key Words: Dental Plaque, Gingivitis, Aloe vera, Chlorhexidine, Gingival Index, Plaque Index.

Introduction

Plaque-induced gingivitis is one of the most frequent periodontal diseases, affecting more than 90% of the population, regardless of age, sex or race. Plaque control and prevention of gingivitis is the main goal of prevention of periodontal diseases [1]. For effective plaque control, several mechanical oral hygiene aids as well as a number of anti plaque agents are available. At present mechanical methods of dental plaque removal are widely regarded as being a highly effective means of helping to control progression of dental caries and periodontal diseases [2]. Mouth rinses are generally considered as adjuncts to oral hygiene and widely used in the delivery of active agents to the teeth and gums. Such agents have been frequently prescribed as adjuvant in the prevention or treatment of oral diseases because they can inhibit bacterial colonization, growth, and metabolism, and consequently interrupt the formation of mature bio-film, changing it at biochemical and ecological levels [3-5].

Chlorhexidine is regarded as benchmark control in plaque removal, but side effects such as staining of the teeth and the tongue, altered taste sensation, and increased calculus formation often deters its use for long periods. Hence, there is a need to develop a naturally occurring, indigenous and cost-effective oral hygiene aid. One such aid could be in the form of *Aloe vera* extract.

Aloe vera is a cactus-like plant, which is a member of the *Lilaceae* family. The mucilaginous tissue in the center of the *Aloe vera* leaf

(*Aloe vera* gel) has traditionally been used for treatment of digestive tract disorders, sunburn, wounds and orally as a laxative. Today, in addition to these uses, aloe is used as a folk or traditional remedy for a variety of conditions, including diabetes, asthma, epilepsy, and osteoarthritis. It is also used topically for osteoarthritis, burns, sunburns, and psoriasis.

Many active ingredients of the *Aloe vera* inner gel have been identified. The gel consists of 98-99% water and the remaining 1-2% contains the active compounds, including aloesin, aloin, aloe-emodin, aloemannan, acemannan, aloeride, naftoquinones, methylchromones, flavonoids, saponin, sterols, amino acids and vitamins. The pharmacological actions of *Aloe vera* gel as studied *in vitro* and *in vivo* include anti-inflammatory, antibacterial, antioxidant, immune-boosting and hypoglycemic properties [6-9].

Although the medicinal use of *Aloe vera* has been reported, not much literature is available regarding its use in the field of dentistry. The purpose of this study was to evaluate clinically the efficacy of *Aloe vera* in preventing plaque accumulation and gingival inflammation.

Material and Methods

This placebo controlled, triple blind randomized control trial; three group parallel study was conducted in the department of public health dentistry on volunteered university students of Teerthankar Mahaveer University. Protocol was approved by the Institutional Review Board

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(IRB) of Teerthankar Mahaveer University. All subjects signed an IRB approved consent form. Pilot study was done on 5 patients in each group to check the feasibility of the study; results are not included in the present study. The study was carried out according to the guidelines of the Declaration of Helsinki [10] for biomedical research involving human subjects.

Inclusion and exclusion criteria

The students with no history of any dental treatment, antibiotic or anti-inflammatory drug therapy for the past 3 months were included in the study. Students with any of history systemic diseases/conditions, fibrotic gingival enlargement and smoking were excluded from the study. Participants with good general health, a baseline plaque score greater than 1.5 and a baseline DMFT index of 2.5 to 5 were included in the study. Those volunteers who had used antibiotics or mouthwash for 5 consecutive days or corticosteroids in the past 30 days were excluded from the study. Those subjects who had a history of sensitivity to any mouthwash or used removable prostheses or an orthodontic appliance were excluded from the study. Those, who had undergone professional measures to remove plaque and calculus in the past 15 days.

Sample size and Randomization

All the undergraduate University students of Teerthankar Mahaveer University were subjected to clinical examination, and a sampling frame (n=1785) was prepared of those who fulfilled the inclusion and exclusion criteria. A sample size of 345 was arrived at by using N Master Statistical Software. A total of 345 volunteers were randomly allocated into the three study groups through computergenerated random numbers. Random allocation of mouth rinses using the lottery method was done. Individuals were identified by code numbers throughout the study.

The total sample size was 345 (115 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 2.359 and standard deviation 2.269. Based on the above calculation the minimum sample required in each group was 115 subjects.

None of the eligible subjects refused to participate in the study.

Group 1 (n=115) was given *Aloe vera* mouthwash and instructed to use 10 ml twice a day for 30 days.

Group 2 (n=115) was given Chlorhexidine and instructed to use 10 ml twice a day for 30 days.

Group 3 (n=115) was the placebo group and distilled water was given as the mouthwash. They were also instructed to use 10 ml of distilled water twice a day as mouthwash for 30 days.

All the three groups followed same oral hygiene instructions, except for the use of allocated mouthrinse. All the three solutions were made of same color with the help of department of pharmacy and kept in coded containers and decoded later. Students in all the three groups were instructed to rinse their mouth with 10 ml of mouthwash twice daily after breakfast and other after lunch for 30 days for one minute and not to rinse with water thereafter. Twice daily rinsing was supervised throughout the week in the department of public health dentistry by a research assistant. Unsupervised rinsing took place on the weekends in the hostel rooms and was documented in a diary completed by the subject. Data was collected three times in 30 days for assessing plaque and gingivitis. Clinical measurements for plaque and gingivitis were taken at baseline (prior to the study), at 15 days and at the end of the study (4 weeks) and were recorded on chart. Study subjects, the investigator/examiner, assistants, data analyser involved in the trial were masked with respect to the treatment allocated. During the thirty days of the trial, the subjects continued to exercise their regular non-supervised, self-performed plaque control measures. All individuals were given a personal set containing the same type of toothbrush and fluoride whitening toothpaste to decrease the possible side effects of staining and lessen examiner bias. No oral prophylaxis was done before the

start of the study. All the enrolled subjects were the volunteers, no monetary help or the payment was given to them. However they were provided with free oral prophylaxis (if needed) at the end of study.

A single examiner, who was trained and calibrated to record the plaque and gingival scores, recorded the findings at all three intervals and for all the three groups. Using kappa statistics, intra-examiner reliability was 0.84 for the gingival index and 0.88 for the plaque index. The recorder was blinded to the type of the mouthwash used by participants.

Preparation of Aloe vera gel

Aloe vera juice and placebo was provided by the *Aloe vera* research group of the Faculty of Pharmacy, Teerthankar Mahaveer University. *Aloe vera* juice consisted of 99% aloe juice, 0.2% preservative, 0.001% lemon-lime flavor, and sweetened with sorbitol. The placebo solution and the control were taste-matched, with identical astringency and consistency [11].

Plaque and gingivitis analysis

Scoring of supra gingival plaque was done with the help of disclosing agent (containing erythrosine dye) by Turesky modification of the Quigley–Hein plaque index [12]. Scoring of gingival inflammation (gingivitis) was done by Gingival Index of Loe & Silness [13].

Statistical analysis

The data were analysed using SPSS version 17. ANOVA followed by post-hoc LSD were used for analysis. P value of 0.05 was taken to be significant.

Results

Attrition of the sample

There was no attrition of sample in all the three groups as all the study groups completed the study.

Plaque and gingivitis score

The mean plaque and gingival scores for the Group I, II, III are depicted in Table 1. ANOVA was used to statistically analyze the reduction in plaque and gingivitis in the three groups. ANOVA (Table 2) was carried out to assess the intra- and inter-group variations for plaque and gingivitis respectively. There was difference in the baseline value in plaque and gingivitis as no subject was given oral prophylaxis before the start of study. There was a statistically significant decrease in the plaque and gingivitis in both the Aloe vera and chlorhexidine groups at 15 days and 30 days (P<0.05) (Tables 2 and 3). There was progressive decrease in the plaque and gingivitis score at 5% level of significance. Chlorhexidine group showed maximum decease as compared to Aloe vera group but difference was not statistically significant. Multiple comparisons were obtained by Post-hoc LSD. The difference in the decrease in plaque (P=1.725 at 15 days and P=1.514 at 30 days) and gingivitis (P=1.093 for 15 days and P=1.103 for 30 days) between Group I and Group II was not statistically significant. However, the difference between Aloe vera and the placebo group and chlorhexidine and the placebo group was statistically significant (P < .05). Data shows that there was no statistical significant difference between Aloe vera based mouth rinse and chlorhexidine for any clinical parameters throughout the study. P >.05 both for plaque and gingivitis in comparing Group I (Aloe vera Group) and Group II (Chlorhexidine) results (Table 4).

Discussion

Prevention of dental caries and periodontal disease can be achieved by proper and regular tooth-brushing, flossing and rinsing with mouthwashes containing antibacterial agents. Chlorhexidine, sodium hypochlorite, cetylpyridinium chloride and amine fluoride are widely used as mouthwashes and irrigating agents that can inhibit the growth of potentially pathogenic oral bacteria. Although these antimicrobial agents are widely used, immediate hypersensitivity

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Plaque Inde	x								
	Group 1		Group 2			Group 3			
	Baseline	15th day	30th Day	Baseline	15th day	30th Day	Baseline	15th day	30th Day
Mean	3.00	2.71	2.49	2.61	2.35	2.52	2.17	2.23	2.60
Gingival Ind	lex					,			
	Group 1			Group 2			Group 3		
	Baseline	15th day	30th Day	Baseline	15th day	30th Day	Baseline	15th day	30th Day
Mean	2.23	1.60	1.35	2.36	1.73	1.44	2.40	2.40	2.50

Table 2: ANOVA of the three study groups.

 Table 1: Mean Plague and Gingival scores for the samples of Group I, II and III.
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		Sum of Squares	Df	Mean Square	F	Sig.
Plaque Baseline	Between Groups	7.290	2	8.56	9.838	.012
	Within Groups	32.145	54	.584	9.030	
Plaque (15 days)	Between Groups	12.906	2	11.45	(019	.001
	Within Groups	62.584	54	.968	6.018	
Plaque (30 days)	Between Groups	22.106	2	21.053	3.529	.001
	Within Groups	92.268	54	1.441	5.529	
Gingivitis Baseline	Between Groups	11.414	2	10.207		.021
	Within Groups	56.963	54	.875	10.952	
Gingivitis	Between Groups	22.906	2	16.975	11.611	.001
(15 days)	Within Groups	82.584	54	1.287	11.011	
Gingivitis	Between Groups	38.106	2	19.161	25 (07	.001
(30 days)	Within Groups	90.268	54	1.404	25.697	

Table 3: Post – hoc LSD test for multiple comparison.

Variable	(I) Group	(J) Group	Std. Error	Sig.
Dia sua Dagalina	Aloe vera	Chlorhexidine Placebo	.15892 .15892	.042 .022
Plaque Baseline	Chlorhexidine	<i>Aloe vera</i> Placebo	.15892 .15892	.042 .036
	Placebo	Aloe vera Chlorhexidine	.15892 .15892	.025 .036
Plaque (15 days)	Aloe vera	Chlorhexidine Placebo	.15695 .15695	1.725 .001
	Chlorhexidine	<i>Aloe vera</i> Placebo <i>Aloe vera</i>	.15695 .15695	1.725 .001
	Placebo	Chlorhexidine	.15695 .15695	.001 .001
Plaque (30 days)	Aloe vera	Chlorhexidine	.15965	1.514
	Chlorhexidine	Placebo Aloe vera Placebo	.15965 .15965	.001 1.514
	Placebo	<i>Aloe vera</i> Chlorhexidine	.15965 .15965 .15965	.001 .001 .001

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

reactions, toxicity, tooth staining and other side effects have been reported. Moreover, it has been reported that chlorhexidine and sodium hypochlorite are cytotoxic to human periodontal ligament cells, inhibit protein synthesis, and affect mitochondrial activity, thus having detrimental effects on vital tissues [13-15].

The natural phytochemicals isolated from medicinal plants used in traditional medicine have been considered useful alternatives to synthetic drugs. Many medicinal plants and their products are widely used for prevention and treatment of oral diseases, and among them *Aloe vera* is of particular interest and has been used therapeutically for a long time [16-19].

This study can be considered complying with the requirements of an ideal mouthrinse protocol as specified by Barnett [20].

Additional inclusion of a positive control enhances the quality of

the study. Even though there are some promising results, the clinical effectiveness of *Aloe vera* is not sufficiently defined at present and warrants further investigations [21] So the present study was designed to determine the anti plaque and anti gingivitis effect of Aleo vera, within the guidelines required by the American Dental Association Council on Scientific Affairs for evaluating the clinical efficacy of chemotherapeutic mouth rinses (American Dental Association Council on Scientific Affairs, 1997).

In the present study treatment doses were applied after the breakfast and after lunch. In order to eliminate the bias decreased flow of the saliva during overnight that could be attributed to was also successful in reducing the plaque index. The students did not discontinue with their routine oral hygiene practices.

Variable	(I) Group	(J) Group	Std. Error	Sig.
	Aloe vera	Chlorhexidine Placebo	.16422 .16422.1	.042 .022
Gingivitis Baseline	Chlorhexidine	<i>Aloe vera</i> Placebo	6422 .16422.1	.042 .036
	Placebo	Aloe vera Chlorhexidine	6422 .16422	.025 .036
	Aloe vera	Chlorhexidine Placebo	.15695 .15695	1.093 .001
Gingivitis (15 days)	Chlorhexidine	<i>Aloe vera</i> Placebo <i>Aloe vera</i>	.15695 .15695	1.093 .001
	Placebo	Chlorhexidine	.15695 .15695	.001 .001
	Aloe vera	Chlorhexidine Placebo	.15527 .15527	1.103 .001
Gingivitis (30days)	Chlorhexidine	<i>Aloe vera</i> Placebo <i>Aloe vera</i>	.15527 .15527	1.103 .001
	Placebo	<i>Aloe vera</i> Chlorhexidine	.15527 .15527	.001 .001

Table 4: Post – hoc LSD test for multiple comparison.

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

Students were instructed to rinse their mouth with 10 ml of solution for a period of 1 min after breakfast and lunch and then were told not to rinse their mouth with water or drink anything for half an hour. Similar amount and duration of mouthwash administration was followed in a study conducted by Axelsson [2]. Chlorhexidine preparation in our study did not contain alcohol. Leyes et al. [22] stated that the alcohol-free rinse was as effective as one containing alcohol in controlling plaque and reducing gingival inflammation.

Despite its commercial use for pharmaceutical purposes, there is no sufficient literature to support anti gingivitis and anti plaque efficacy of *Aloe vera* mouthwash. The antimicrobial efficacy of *Aloe vera* was evaluated in a preliminary *in vitro* study exposing supragingival plaque samples to different concentrations of *Aloe vera*. *Aloe vera* at 100% concentration was found to have maximum effect *in vitro*. In this study *Aloe vera* at 100% concentration was used. The present study is the first reported trial on *Aloe vera* with large sample size and following ADA guidelines on Scientific Affairs for evaluating the clinical efficacy of chemotherapeutic mouth rinses.In the present study there was no difference between chlorhexidine and *Aloe vera* mouthwash when compared against plaque and gingivitis. Findings of Villalobos et al. [23] and Chandrahas et al. [24] differed from our study may be due to different concentration of mouthwash used and different study design used.

Only three studies [25-27] have been conducted to check the effect of dentifrice containing *Aloe vera* on plaque and gingivitis; and have shown a significant reduction in plaque and gingivitis.

The antimicrobial effect of *Aloe vera* has been demonstrated in an *in vitro* study by Lee et al. [28] where it was reported to inhibit the growth of diverse oral microorganisms such as *Streptococcus mutans*, *Streptococcus sanguis*, *Actinomyces viscosus*, and *Candida albicans*. The low plaque scores at the conclusion of the study could be attributed to the antibacterial properties of *Aloe vera*.

Aloe vera resulted in significant reduction in gingival index, which can be attributed to its anti-inflammatory, antibacterial, wound healing properties. *Aloe vera* has numerous anti-inflammatory agents. Carboxypeptidase present in *Aloe vera* inactivates bradykinin thereby reduce prostaglandin synthesis and inhibits oxidation of arachidonic acid, which might decrease inflammation and relieves pain.

Magnesium Lactate present in *Aloe vera* inhibits Histidine Decarboxylase, thereby preventing the formation of histamine from

histidine in mast cells. The decrease in gingival index can also be attributed to presence of sterols as anti-inflammatory agents and lupeol as an antiseptic analgesic [29].

Aloe vera inhibits the stimulated granulocyte Matrix Metallo Proteinases (MMPs) inhibiting cyclooxygenase and lipo-oxygenase pathways and blocks polymorpho nuclear leucocyte, thus decreases edema [30,31].

Aloe vera inhibits the production of free oxygen radicals by activated Polymorpho Nuclear Leucocytes (PMNs). It has also shown to provide relief in swelling, bleeding gums and is an antiseptic for pockets and antifungal for thrush [32]. Mannose-6-phosphate is a major structural constituent of *Aloe vera*. It is responsible for healing by *Aloe vera*, and was found to be accompanied by higher levels of hyaluronic acid and dermatan sulfate, which were suggested to stimulate collagen synthesis and fibroblast activity [33]. Vitamin C present in *Aloe vera* is involved in collagen synthesis, increases concentration of oxygen at the wound site because of dilation of blood vessels. *Aloe vera* is also shown to provide relief in swelling, bleeding gums and is an antiseptic for pockets and antifungal for thrush [32].

The test groups showed significant reductions on plaque and gingivitis at the end of the trial (*Aloe vera*, 40%; CLX, 52%); this is consistent with previous studies. Nevertheless, this percent difference was not significant, showing that *Aloe vera* had a potential similar to chlorhexidine as an anti plaque and antigingivitis agent. Noskova, [34] used *Aloe vera* to treat early stages of periodontitis and got good results.

Participants of clinical trials may experience some improvement not specifically associated to the therapeutic properties of the test agent, but rather related to a behavioural change; this is known as the Hawthorne effect. Subjects enrolled in oral hygiene studies usually improve their tooth brushing irrespective of the product they receive [35].

Although the volunteers in the present study were not aware of which mouthwash they were using, another main factor is the so-called novelty effect, which is the motivation to improve oral hygiene practices induced by the use of a new substance. In contrast, lack of compliance with correct use of the mouthwash can occur as well. In order to minimize these potential, participants were asked to rinse in front of the investigator and to get back the bottles which were given to them for holiday rinsing, so we could perform direct and indirect assessment of compliance.

Conclusion

In this study, *Aloe vera* has stood the test by demonstrating similar effect on plaque and gingivitis when compared with the bench mark control Chlorhexidine.Our results provide practical hints for better management of plaque and gingivitis, particularly in patients who prefer to use herbal medicine instead of synthetic drugs. Results of this study clearly favors promotion of *Aloe vera* as a mouth rinse

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among communities especially belonging to low socioeconomic strata as *Aloe vera* is easily accessible and is cheap and safe alternative to chlorhexidine. Further studies in other populations with large sample size and with more duration of follow-up are necessary to confirm our results.

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