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EFFECT OF ALOVERA MOUTH WASH ON COMMON MICROBIAL FLORA OF ORAL CAVITY

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Abstract

Introduction: Aloevera is popularly known as Aloe barbadensis by taxonomists. It is being used since 1750 BC by Mesopotamians and Egyptians. This is awell-known medicament.

Aim: The aim of the study was to evaluate clinically the efficacy of the Alovera mouthwash on common microbial flora of oral cavity.

Materials and Method: This was a double-blind, randomized, parallel study design. 243 subjects were randomly assigned in three groups. Three groups were 99% Alovera mouthwash group, 0.12% Chlorhexidine gluconate group and distilled water group respectively. 0.12% Chlorhexidine gluconate was taken as positive control, whereas distilled water negative control. In each group 81 subjects were taken. Interventions consisted of 30 days therapy. The subjects were supervised to rinse approximately 15ml of mouth rinse for 30 seconds, twice per day for 30 days. The microbial assessment for saliva responsible for dental caries and periodontal disease was assessed prior and after treatment. Data were statistically analyzed by Statistical Package for Social Sciences (SPSS) 22 version developed by IBM Corporation. For the intra group comparison paired t-test was used and for the intergroup comparison unpaired t-test was used. The P value was set at <0.05.

Results: The data shows that a mouthrinse based on the Alovera is equally effective in reducing microbial flora responsible for dental caries and periodontal disease.

Conclusion: Microbial analysis indicates that 99% Alovera mouth wash has significant role in reducing the common oral mcro-flora responsible for dental caries and periodontal diseases.

Keywords: Alovera, Chlorhexidine, Mouth rinse, Oral flora.

Introduction

Dental caries and periodontal disease are recognized as a major public health problem worldwide.[1] In developing countries like India the prevalence of these diseases is on the rise. As the treatment is expensive and requires a lot of manpower, prevention at the primary level is the solution of choice. Biofilm, dental plaque and its constituent micro-organisms have been implicated in the initiation and progress of caries and periodontal disease. Hamanda and Slade have stated that in the development of plaque, attachment of *Streptococcus mutans* to the surfaces of teeth and their subsequent colonization on human tooth surfaces with *Streptococcus mutans* and *Lactobacillus acidophilus* is necessary for the occurrence of dental caries.[2] All surfaces of the oral cavity, including all tissue surfaces as well as surfaces of teeth and fixed and removable prosthesis and restorations, are coated with glycoprotein pellicle. The pellicle is derived from components of saliva and crevicular fluid, as well as from bacterial and host tissue cell products and debris or food particles. The initial microorganisms colonizing the pellicle- coated tooth surface are predominantly gram-positive facultative microorganisms such as *Actinomyces viscosus*, *Streptococcus sanguis* etc. The plaque mass then matures through the growth of attached species, as well as the colonization and growth of additional species. Preventing these initial micro-organisms from establishing on the tooth surface, can prevent dental caries and periodontal disease and reduces its occurrence. The most effective method of prevention and maintenance of dental caries and periodontal disease is utilization of antimicrobial mouth rinses adjunct to mechanical oral hygiene measures.[3]

Aloevera is a well-known medicinal plant belonging to the *Liliaceae* family. It is a cactus-like plantthat grows readily in hot dry climates. The mucilaginous tissue in the centre of the Aloevera leaf has traditionally been used for treatment of digestivetract disorders, sunburn and wounds. To date, more than75 active ingredients of the Aloevera inner gel have been identified. The gel consists of 98-99% water andthe 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 levels of these compounds in Aloe plants are highly variable according to species and strain, as well as growth conditions. The pharmacological actions of Aloevera gelas studied *in vitro* and *in vivo* include anti-inflammatory, antibacterial, antioxidant, immune-boosting and hypoglycaemic properties.[4]

Hence the aim of this study is to evaluate the efficacy of the Alovera mouthwash on common oral micro-flora.

Materials and Method

Study design: This was a double-blind, randomized, parallel study design.

Inclusion criteria

- Subject should have frank carious cavities DMFT ≥3.
- Definite periodontitis CPI ≥2.
- Free from any systemic diseases

Exclusion criteria

- Subjects using antibiotics or antiseptic mouthwashes for at least 3 months prior to the study and at the time of study.
- Undergoing any dental treatment or with extensive intraoral prosthesis.
- Using Alovera in any form as an oral hygiene aid.

Participants

Nursing students were selected for the study for the ease of obtaining study subjects and their supervision, as well as the environment in which all the subjects resided matched and was controlled.

Study Settings

A special format was designed exclusively for recording pertinent general information and observed findings. The investigator was trained and calibrated in the Department of Public Health Dentistry of the institution. This study was conducted in the Department of Public Health Dentistry in collaboration with the Department of Microbiology.

Ethical clearance was obtained from Institutional Ethical Committee. A written informed consent was obtained from all students selected for this study.

A pilot study was conducted on 30 subjects to get the feasibility and acceptability of the study.

Preparation of test solution

Aloevera juice was provided by the Aloevera research group of the Faculty of Pharmacy. Aloevera juice consisted of 99% Aloevera juice, 0.2% preservative, 0.001% lemon-lime flavour, and sweetened with sorbitol.[5]

Intervention

Study subjects were randomly allocated in three groups:

Group I was 12% Chlorhexidine gluconate (positive control) group.

Group II was 099% Alovera group.

And Group III was Distilled water (negative control) group.

Group I received treatment with 0.12% Chlorhexidine gluconate. Group II received treatment course of 99% Alovera mouthwash. And Group III treated with distilled water. In each Group 81 subjects were allocated. Detail flow chart of the protocol is given in Figure 1. Saliva samples of all subjects were collected prior to the study and send for microbiological analysis. To reduce inter-observer bias, all saliva samples were collected by single person. After the baseline examination, subjects were instructed to rinse approximately 15ml of respective oral rinses for 30 seconds twice a day for the period of 30 days. Subjects were provided with a supply of coded mouth rinse and plastic dosage measuring cups. The investigator and assistant involved in this trial were blinded with respective mouthwashes. The saliva samples were collected after 15 days and 30 days of intervention. During the study, subjects followed their usual oral hygiene and dietary habits and were instructed to refrain from using other mouth rinses.

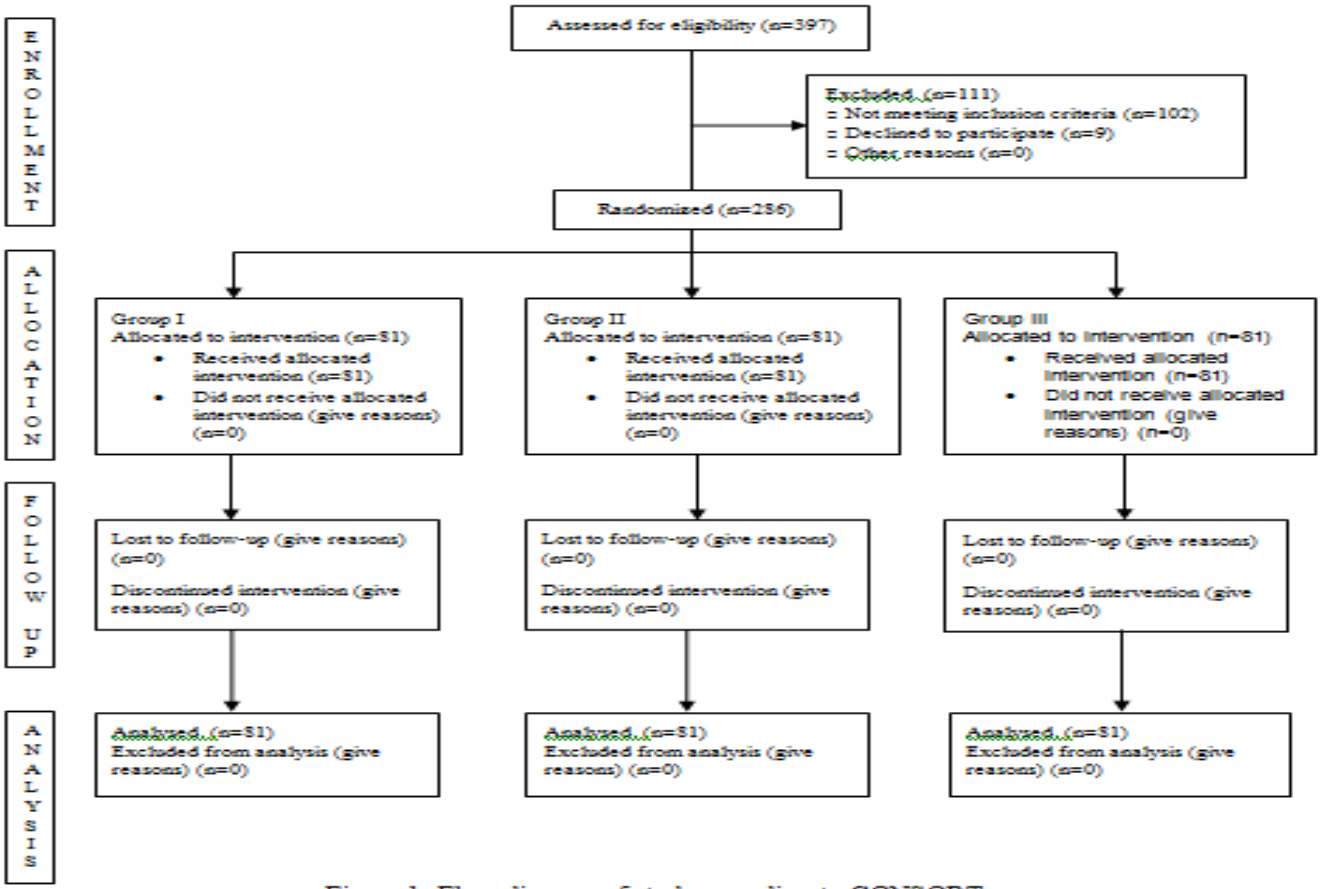

Figure 1: Flow diagram of study according to CONSORT

Sample size

Sample size was calculated using the standard formula: $n = z^2 [p(1-p)]/e^2$; where n = size of sample; p = approximate prevalence rate; z = critical value at a specified level of confidence; e = difference between sample proportion and population proportion.

As per the above mentioned formula and result of pilot study a minimum sample of 271 nursing students was obtained. Few extra subjects were included in this study and the final sample size was 286.

Randomization

Research coordinator randomly assigned eligible subjects in three groups by lottery method. Study investigator, research coordinator and participating subjects were blinded to treatment allocation.

Microbiological analysis

Four separate strains were used for *Streptococcus mutans*, *Lactobacillus acidophilus*, *Actinomyces viscosus* and *Streptococcus sanguis.* These micro-organisms are initial colonizers in the dental plaque and could induce dental caries and early gingivitis. For recording the count of *Streptococcus mutans*, *Lactobacilli acidophilus*, *Actinomyces viscosus* and *Streptococcus sanguis* stimulated saliva was used. Subjects were asked to stimulate salivary flow by chewing action with sterile cotton gauge. This procedure was carried out because clearing the oral cavity with any residual unstimulated saliva.

The subjects then instructed to chew sterile cotton gauge for next four minutes for secretion of a pool of stimulated saliva in oral cavity. At the end of four minutes, the subjects were made to expectorate that stimulated saliva in to sterile penicillin vials. Then that stimulated saliva was transported to the microbiology department within 30 minutes. A semi-quantitative i.e. 4 quadrant streaking method was adopted. Using a standard loop the saliva was streaked onto:

- Mitissali varius with bacitrac in agar enriched with sucrose 15% for *Streptococcus mutans*
- *Lactobacillus MRS agar* for *Lactobacillus acidophilus*,
- 3% sucrose media for *Streptococcus sanguis*
- GMC media for *Actinomyces viscosus*

The media was prepared according to the manufactures instructions (Hi-media Company, Institute of Microbial Technology IMTECH, Chandigarh, India).

The media were then incubated in Dynox charge microjars at 37^0C, under the aerobic and microaerophilic conditions for 5 days. The growth in all the four quadrants was recorded. The colonies were identified based on colony morphology of gram staining. The growths of microorganisms were recorded quadrant wise.

Statistical analysis

Data were statistically analyzed by Statistical Package for Social Sciences (SPSS) 22 version developed by IBM Corporation. For the intra group comparison paired t- test was used and for the intergroup comparison unpaired t- test was used. The P value was set at <0.05.

Results

The growth of *S. mutans, L. acidophilus, A. viscosus and S. sanguis* at different time intervals with intra-group comparison is given in Table No: 1.

Table No-1: Growth of S. Mutans, L. Baccilus, A.Viscosus and S. Saguis at Different Time Intervals with Intra-Group Comparison.

Group	Strains	Pre-rinse Mean±SD	Post-rinse (15days) Mean±SD	Post-rinse (30days) Mean±SD
Chlorhexidine (n=81)	S. mutans	3.10 ± 0.76	0.0 ± 0.0 **P<0.001***	0.45 ± 0.62 **P<0.001***
	L. acidophilus	3.63 ± 0.49	1.83 ± 0.46 **P<0.001***	2.80 ± 0.41 **P<0.001***
	A. viscosus	3.53 ± 0.51	1.67 ± 0.47 **P<0.001***	2.73 ± 0.52 **P<0.001***
	S. saguis	3.73 ± 0.45	1.80 ± 0.55 **P<.001***	2.80 ± 0.55 **P<.001***
Alovera (n=81)	S. mutans	3.40 ± 0.09	0.27 ± 0.45 **P<0.001***	1.60 ± 0.09 **P<0.001***
	L. acidophilus	3.76 ± 0.43	1.73 ± 0.45 **P<0.001***	2.93 ± 0.74 **P<0.001***
	A. viscosus	3.70 ± 0.47	2.07 ± 0.64 **P<0.001***	3.60 ± 0.50 **P<0.83**
	S. saguis	3.67±0.41	1.97±0.61 **P<0.001***	3.63±0.49**P<0.75**
Distilled Water (n=81)	S. mutans	3.51 ± 0.08	3.42 ± 0.59 **P<0.87**	3.41 ± 0.57**P<0.86**
	L. acidophilus	3.66 ± 0.54	3.63 ± 0.65 **P<0.93**	3.61 ± 0.67 **P<0.94**
	A. viscosus	3.67 ± 0.38	3.55 ± 0.68 **P<0.87**	3.52 ± 0.59 **P<0.84**
	S. saguis	3.67 ± 0.41	3.61 ± 0.87 **P<0.91**	3.59 ± 0.79 **P<0.88**

*Highly significant

In Group I, using Chlorhexidine mouthwash all the subjects showed significant reduction of *S. mutans*, *L. acidophilus*, *A. viscosus* and *S. sanguis* at 15days [p=0.001] from the baseline values i.e. before rinsing. Similarly at the 30 days [p=0.001] a significant difference was found indicating that of *S. mutans*. *L. acidophilus*, *A. viscosus* and *S. sanguis* were inhibited by the first group over a long time.

In Group II, using Alovera mouthwash all the subjects showed significant reduction of *S. mutans*, *L. acidophilus* at 15 days [p=0.001] from the baseline values i.e. before rinsing. Similarly at the 30 days non significant reduction for *A. viscosus* [p=0.083] and S. *sanguis* [p=0.75] indicating that *A. viscosus* and S. *sanguis* was not inhibited by this group over a long time. In Group-III, using distilled water all the subjects showed no significant reduction of *S. mutans*. *L. acidophilus*, *A. viscosus* and *S. sanguis* at 15 days [p=0.16] and 30 days [p=0.16].

Intergroup comparison

Streptococcus mutans (Table No: 2)

15 days significant difference was observed in Chlorhexidine vs Alovera group. This suggested that this group shows a significant reduction in the count of streptococcus mutans. At 30 days change is maximum in Chlorhexidine group than in Alovera group and is minimum in the distilled water group.

Table No-2: Intergroup Post Rinse Comparison Streptococcus Mutans Growth.

Group	P-Value (15days)	P value (30days)
Group I Vs Group II	**< 0.01***	**<0.001****
Group I Vs Group III	**<0.001****	**<0.001****
Group II Vs Group III	**<0.001****	**<0.001****

*Significant

**Highly significant

Lactobacillus acidophilus (Table No: 3)

At 15 days change is maximum in Alovera Group than the Chlorhexidine Group and was minimum in the distilled water group. At 30 days there is no significant change in Chlorhexidine vs Alovera group. In Chlorhexidine vs distilled water and Alovera vs distilled water groups change is more in Chlorhexidine and Alovera than the distilled water group.

Table No-3: Intergroup Post Rinse Comparison of Lactobacillus Acidophilus Growth.

Group	P-Value (15days)	P value (30days)
Group I Vs Group II	< 0.03	**<0.001***
Group I Vs Group III	**<0.001***	**< 0.001***
Group II Vs Group III	**<0.001***	**<0.001***

*Highly significant

Actinomyces viscosus (Table No: 4)

At 15 days in Chlorhexidine vs Alovera Group change is more in Chlorhexidine than Neem but the difference is not significant [p=0.09]. In Chlorhexidine vs distilled water and Alovera vs distilled Water groups change is significant and is higher in Chlorhexidine and Alovera group than the distilled water. At 30 days change is significantly higher in Chlorhexidine than Alovera and distilled water and non significant difference in Alovera and distilled water.

Table no-4: Intergroup Post Rinse comparison of Actinomyces Viscosus Growth.

Group	P-Value (15days)	P value (30days)
Group I Vs Group II	**<**0.09	**<0.001***
Group I Vs Group III	**< 0.001***	**<0.001***
Group II Vs Group III	**<0.001***	**<**0.84

*Highly significant

Table no-5: Intergroup Post Rinse Comparison of Streptococcus Sanguis Growth.

Group	P-Value (15days)	P value (30days)
Group I Vs Group II	**<**0.04	<0**.001***
Group I Vs Group III	<0**.001***	<0**.001***
Group II Vs Group III	<0**.001***	**<** 0.40

*Highly significant

Streptococcus sanguis (Table No: 5)

At 15 days in Chlorhexidine vs Alovera group change is maximum in Chlorhexidine than in Alovera and is minimum in distilled water group. At the 30 days in Chlorhexidine vs Alovera and Chlorhexidine vs distilled water change is more in Chlorhexidine than Alovera and distilled water. In Alovera vs distilled water there was no significant change.

Discussion

Alovera is one of the most widely researched tropical trees, with almost all its parts being put for variety of use.

Effect on Streptococcus mutans

The findings indicate that the Alovera inhibited the growth of S. mutans. Similar results were obtained by Fani M & Kohanteb J (2012).[6] the same properties could be attributed to the growth inhibition of S. mutans in the present study. The inhibitory effect of Chlorhexidine observed in the study has also been well documented by Kingsman A *et al.* (1988).[6] the reason for isolating streptococcus mutans in the study is that they are the most common and important Streptococcus species for the initiation of caries.

Effect on Lactobacillus acidophilus

Lactobacilli are considered to be commonly associated with progression of caries lesion Houte JV (1980)[7], hence there counts were included to determine the effect of mouth washes on the progression of lesion. Two major factors are said to cause increasing oral lactobacilli counts: exposed carious lesions and frequent intake of sugars as stated by Sreebny LM (1982)[8]. Since no diet counseling was imparted, neither was any intervention done but still there was reduction in lactobacilli counts in the Alovera group and the Chlorhexidine group could be that these antimicrobials can penetrate deep into the carious lesions thereby interacting with lactobacilli.

Effect on Actinomyces viscosus

Though A. viscosus is considered to be the main bacteria for root caries formation, they are one of the initial colonizers of plaque formation Fejerskov *et al.* (1993).[9] the count of A. viscosus is increased in patients suffering from gingivitis. Hence there count is recorded as an indicator of existing early periodontal disease. The findings indicate that the Chlorhexidine inhibited the growth of A. viscosus. In Alovera group all subjects show significant reduction at 15 days and non significant reduction at 30 days. A similar finding was observed in the study of Oliveira SMA (2008).[10]In distilled water group there was no reduction in the account of A. viscosus.

Effect on Streptococcus sanguis

Axellson *et al.* (1987)[11] have stated that S. sanguisone of the constituting bacteria of the mutans streptococci group-or among the initial colonizers of plaque that plays a role in the formation of caries and gingivitis as well. Hence S. sanguis was included in the bacterial assay of the study. Also the primary colonizers of dental plaque of the free gingival margin are considered to be predominately gram positive and cocci in nature which are responsible for the initiation of gingivitis.[12]

Chlorhexidine group all subjects showed significant reduction in S. sanguis count. Alovera group all subjects showed significant reduction at 15 days and no significant reduction at 30 days. Distilled water group the subjects showed no significant reduction in the microbial count. In the inter group comparison change is maximum in Chlorhexidine group than in Alovera group and is minimum in distilled water group.

Conclusion

Microbial analysis indicates that 99% Alovera mouth wash has significant role in reducing the microbial flora of responsible for dental caries and periodontal diseases. The results may have an important impact in order to create an effective and inexpensive oral health intervention in low-socioeconomic communities.

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