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The Plaque Inhibitory Effect of Aloe Vera Mouthrinse in A Four Day De Novo Plaque Formation Model- A Randomized, Double Blind Crossover Study

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Abstract: *Mechanical oral hygiene procedures are most widely accepted procedures for controlling bacterial plaque, but these methods have certain limitations. Based on several clinical trials, chlorhexidine mouthrinse have been accepted as a benchmark to be used as an adjunct to mechanical oral hygiene aids, however it is associated with side effects in its long term use. The present study was undertaken to evaluate the antiplaque efficacy of a herbal Aloe vera mouthrinse and compare it to gold standard Chlorhexidine. A randomized, double blind, placebo-controlled, three group crossover, four day de novo plaque accumulation model was used. Study involved three experimental phases with a ten day washout period in between each phase. Twelve student volunteers were refrained from all oral hygiene measures for four days, but rinsed twice daily with 10 ml of one of the randomly assigned mouthrinse (chlorhexidine, Aloe vera, and placebo mouthrinse) in each test phase. Plaque index and plaque area was measured on day four. The Aloe vera mouthrinse showed a significant inhibition of plaque re-growth compared to placebo ($P<0.0001$), but the lowest values of plaque index and plaque area were obtained with chlorhexidine. The study concluded that Aloe vera has a promising plaque inhibitory potential but is not as efficacious as chlorhexidine in preventing plaque re-growth.*

Key words: *Aloe vera, chlorhexidine, cross over studies, dental plaque, mouthwashes*

I. Introduction

Dental plaque is an adherent bacterial biofilm that forms on hard and soft tissues intra-orally. [1] The mainstay in preventing periodontal disease is the control of dental plaque. While mechanical methods of plaque removal are considered as standard for individually applied oral diseases preventive practice,the higher prevalence of gingival diseases has prompted research into development of adjunctive methods for controlling oral biofilms. International Association for Dental Research (IADR) supports the benefit of oral rinsing with chemotherapeutics as an adjunct for controlling plaque and maintaining gingival health. [2]

Various synthetic chemical agents have been evaluated over the years with respect to their antimicrobial activity in the oral cavity, but none are without shortcomings. Chlorhexidine digluconate, currently recognized as gold standard has its duration of use limited to just a few weeks because of undesirable side effects such as tooth discoloration, altered taste, and less commonly desquamation of oral mucosa. [3]Hence, the quest for a long term, ideal and a safe antiplaque and antigingivitis agent continues and this has also encouraged the search for alternative agents based on herbal extracts.

Many medicinal plants and their products are widely used for prevention and treatment of oral diseases, and among them Aloe vera extract can be one such oral hygiene aid which is naturally occurring, indigenous, cost effective with good therapeutic properties. Aloe vera is a cactus like plant, which is a member of Lilaceae family. The gel consists of 98-99% of water and remaining part comprises of pharmacological active compounds. Various uses of Aloe vera in dentistry can be found in literature owing to its multiple properties like anti-inflammatory, antibacterial, antiviral, pain relieving, wound healing, moisturizing and antioxidant potential .[4-6]

On the basis of these researches, the aim of the present study was to evaluate the plaque inhibitory efficacy of Aloe vera mouthrinse in a four day de novo plaque accumulation model by a crossover study design and to compare it with benchmark antiplaque agent chlorhexidine.

II. Materials and Methods

2.1 Study population

Fifteen student volunteers (eight males and seven female students; age range from 19 to 25) participated in the study. The volunteers had a minimum of 25 scorable teeth and documented good standards of oral hygiene and gingival health [papilla bleeding index (PBI) of less than 30%]. The presence of grossly carious teeth, more than one full coverage restorations, fixed or removable orthodontic appliances, poor oral hygiene pocket depth > 5mm or attachment loss > 2mm, known intolerance or allergy to mouthrinses and use of

antibiotics, medications or any other mouthwash in last three months that might interfere with plaque formation were the exclusion criteria.

All eligible volunteers were given an oral and written information about the purpose of the study and were asked to sign an informed consent. The study was conducted in accordance with ethical principles originating in the declaration of Helinski and consistent with clinical practice. The study protocol was approved by The Institutional Ethical Committee of Jamia Millia Islamia before commencing the study.

Test solutions

Test solution 1: Aloe vera mouthrinse (ALV)**:** Two parts of commercially available 98 % pure Aloe vera juice (AVG Ltd.) was diluted with one part of distilled water (2:1). A colorant and flavouring agent was added to make it resemble to Chlorhexidine mouthrinse

Test solution 2: Chlorhexidine mouthrinse (CHX; functional positive control): A commercially available non-alcoholic 0.2% Chlorhexidine mouthwash (Rexidin mouthwash; Indoco remedies Ltd.).

Test solution 3: Placebo mouthrinse (PM; negative control): Distilled water. A colorant and flavouring agent was added to make it resemble to Chlorhexidine mouthrinse

Study design

A randomized, double blind, placebo-controlled and a three group crossover design was used for present clinical study. A total of 45 bottles were dispensed (15 of each mouthrinse) and each bottle was randomly assigned a number from 1 to 45. Randomization was performed by computer generated numbers. All the bottles were identically packed so that they could only be identified by numbers. Dispensing and allocation of mouthrinse was carried out by a person not directly involved in the `study.

Study included three experimental phases, each of a four day trial with a crossover interval of ten days in between each phase. All volunteers received one test rinse out of the three in each experimental phase according to an assigned random order of ALV, PM and CHX mouthrinse. In the first test phase, at baseline (Day one), all volunteers underwent an oral soft and hard tissue examination. Following this plaque was visualized using disclosing solution (2-TONE), and then a thorough scaling and polishing was performed to remove all plaque, stain and calculus, using ultrasonic scalers and hand instruments. To ensure that all deposits are removed a second episode of application of disclosing solution was carried out after which remaining plaque was removed. All normal oral hygiene procedures like brushing, flossing, inter dental brushes and consuming chewing gum were suspended for the next four days, and subjects were instructed to rinse two times a day, before breakfast and in the night for one minute with 10 ml of their assigned rinse. Along with each bottle of rinse, a 10 ml measure was given to facilitate the correct dosage. On Day five, the subjects were recalled along with their bottles so that their compliance can be assessed by measuring the residual mouthwash in them. All the subjects received a re-examination of their oral soft and hard tissue and were scored and photographed for assessment of plaque.

A washout period of ten days was instituted to exclude a carryover effect of the experimental mouthwash. During this time subjects resumed their oral hygiene procedures; following this washout period all volunteers again underwent a session of scaling in order to get plaque index to zero and protocol was repeated using the randomly assigned second test rinse. In a similar fashion third test phase was also carried where same volunteers used their assigned third experimental mouthrinse.

2.2 Clinical evaluation

The PBI by Saxer and Muhlemann was assessed at the buccal sites of the gingiva of all teeth on day one and day five as a control parameter to evaluate the gingival health of the subjects during the whole test period.[9] To test the influence of the experimental mouthrinses on plaque re-growth, clinical parameters recorded were plaque index (PI) according to Turesky et al.[7] modification of Quigley Hein PI [8] (6 point scale with scoring from 0 to 5) and plaque area (PA). For PI, the scores were taken at six surfaces per tooth: mesio-, mid-and disto- buccal, and mesio-, mid-,and disto-lingual after staining plaque with disclosing solution. All teeth except 3rd molars were examined, and all scorings were carried out by the same investigator who was unaware of the allocation of the mouthrinse to the participants. In addition, any adverse effects during the use of mouthrinses were recorded on a question sheet on a scale from absent, mildly, moderately or severely present.

2.3 Plaque area (PA) analysis

Plaque area was evaluated on day five by calculating the percentage of plaque-covered area to total tooth area. After staining with disclosing solution, digital standardized orthoradial photography and computer-based calculation were performed. For this study, only the upper right and left lateral incisors were selected. The stained buccal surface was highlighted on the digital photograph using the Adobe photoshop CS5 extended (12.1 · 3.2 version), and then the number of pixels within the area was calculated. In addition, the circumference of whole tooth surface was also highlighted and the number of pixels within this area was calculated. The

relation between the plaque covered labial area (number of pixels) and the total vestibular labial tooth surface (number of pixels) gave the percentage of existing plaque.[10, 11]

Statistical analysis

After the test period was completed, the evaluation was performed using the computer program Statistical Package for Social Sciences (SPSS) Version 15.0 (SPSS Inc., Chicago, IL, USA) and Lead Tools_ 1991–2000 (LEAD Technologies Inc., Chicago, IL, USA).The mean values of clinical parameters (PI and PA) were calculated for each rinse solution. First analysis of variance (ANOVA) was performed to determine the differences among products tested. In the presence of significant differences, pair wise comparisons were made via Tukey HSD. The Tukey HSD was used as the post hoc test to control the possibility of alpha-error owing to smaller sample size. The confidence level of the study was kept at 95%; hence, a 'P' value < 0.05 indicated statistically significant differences.

Results

3.1 Compliance

Three out of fifteen participants were dropped out of study as they did not follow mouthrinse regimen as instructed. Final analysis was based on observations from remaining twelve participants (six males and six females). With the aid of questionnaire, it was observed that all the rinsing solutions were accepted by the participants, with report of no adverse side effects.

3.2 Plaque growth inhibition

The distribution of mean PI and PA of the three mouthrinses after four days of de novo plaque formation was statistically significant (P<0.001; Table 1).The positive control (CHX solution) attained the lowest value for plaque parameters (PI: 2.84, PA: 38.16%) while the highest was achieved by PM/negative control (PI: 3.83, PA: 58.68%). Mean PI (3.48) and mean PA for ALV (51.42%) was in between the other two test rinses. Statistical significant difference (P<0.0001) in plaque parameters (PI and PA) was observed when CHX was compared to ALV and PM, and ALV to PM (Table 2).

III. Discussion

The incorporation of broad spectrum antibacterial mouthrinses as an adjunct to patient's daily oral hygiene regimens has assumed greater importance with the recognition that most individuals are unable to consistently maintain adequate levels of plaque alone using mechanical methods.[12] As the various synthetic antimicrobial agents are associated with adverse effects like tooth staining, taste alteration, immediate hypersensitivity, toxicity and increased resistance; alternative medicines may be developed from medicinal plants to replace synthetic drugs. The present study was undertaken to compare the plaque inhibitory potential of extract from a medicinal plant Aloe vera with a synthetic benchmark control 0.2% CHX. Additionally a negative control (PM/distilled water) was added to enhance the quality of study.

Present study can be considered complying with the study design protocol for investigating the effect of mouthrinses stated by Lorenz et al.[10] As prospective randomized controlled clinical trials (RCT) provide a higher level of evidence for chemotherapeutic agents used for chemical plaque control, it was adopted in the current study.[13] The four day plaque re-growth crossover study design was chosen for the present endeavour as it has been employed in numerous investigations and can be described as an established method for assessing the plaque inhibitory activity of formulation per se and determines the relative efficacy of different formulations.[10,14-17]. The study design measures the plaque re-growth under the influence of test solution from a zero plaque baseline and avoids confounding influence of tooth brushing, which is highly variable in between individuals. If no plaque inhibition can be seen in this type of study, no further effect of rinsing can be expected in studies where oral hygiene is performed.[14]

Unlike previous plaque associated studies on ALV, present study is the first one to follow a crossover design instead of parallel as it is more standardized and recommended method for a four day plaque re-growth study.[17-22] A particular advantage of a crossover design is that each subject serves his or her own control so it reduces the influence of confounding that arises because of individual variables that effect plaque formation like the salivary flow and composition, existing plaque retention sites, pre-existing gingivitis, dietary habits, and the composition of pellicle.[23,24] Also optimal crossover designs are statistically significant so require fewer subjects than non crossover design. To decrease the "carryover effect" between treatments encountered in crossover trials, a 10 day wash out period was followed in our study. Student volunteers were chosen for the current study as they are well educated, cohesive, and more sensitized to oral hygiene protocols and would understand and comply in a better way to treatment regimens.

In this study, the antiplaque action was assessed by two quantitative parameters PI and PA. We found a statistically significant difference between the plaque index scores of CHX, ALV and PM after a period of four days (Table 1).Though the plaque reduction by ALV (PI: 3.48) was less than CHX (PI: 2.84), it nonetheless

indicates that Aloe Vera can also effectively reduce experimentally induced plaque accumulation. To our knowledge there is only one previous study where the antiplaque efficacy of ALV was studied on four day plaque re- growth model; [17] but unlike ours, it wasn't based on a crossover but on a parallel study design. The result differed from current endeavour as though ALV showed plaque reduction in their study, but a statistical significance wasn't reached. A similar trend was also observed by Karim et al in a clinical trial conducted without abstaining daily tooth brushing.[22] These authors observed plaque reduction by ALV, but with no statistical significance. Results of clinical trials by Chandrahas et al and Villalobos et al were comparable to the present study.[18,20] Chandrahas et al found a significant plaque score reduction by ALV, [20] however their study was based on cessation of tooth brushing only in a specified area and plaque was evaluated after seven days. Oliveria et al and Pradeep et al found a significant improvement in plaque and gingivitis score by using a dentifrice containing ALV.[19 , 21]

In the current study, pattern for PA measurement was similar to PI (Table 1).The mean PA for ALV (PA: 48.20%) was in between that for positive (PA: 38.16%) and negative control (PA: 55.17%).A statistically significant suppression of de novo plaque formation was shown by ALV as compared to PM ($P<0.0001$; Table 2), again suggesting that ALV does have a potential plaque inhibitory activity but less than CHX. As the present study is the first one to evaluate plaque area in a clinical trial for ALV, the results cannot be compared with the previous studies. Quirynen & Van Steenberghe considered the different plaque indices to be not exact enough and used also the plaque area calculation for a more detailed and précised analysis of plaque growth for a period of 96 hours.[25] For evaluation a planimeter was used by these authors. The area of plaque was given as a percentage of the total buccal surface just like it was done in the present study. We used a computer program to calculate the percentage of the plaque covered teeth surfaces from digital photographs similar to Söder et al. and Eaton et al. [26, 27]. Further, Renton-Harper et al. stated advantage of recording digital photographs as they are permanent records that can be re-evaluated at any point of time, and can later be transferred into other index scores; which helps in an easier comparison with other studies.[28]

The plaque inhibitory potential of ALV, demonstrated in the present investigation can be attributed to its antibacterial property. The bactericidal activity of ALV is due to a numerous pharmacological active compounds including anthraquinones, alonin, aloe-emodin, aloetic acid, anthracine, aloe mannan, aloeride, antranol, chrysophanic acid, reistanol, and saponin.[29]Alonin and aloe-emodulin are the major anthraquinones in aloe plant, which have polyphenolic structures that can inhibit protein synthesis by bacterial cells;thus explaining their strong direct antibacterial and anti-inflammatory properties.[30,31] Another component acemannan has been considered to exert an indirect bactericidal effect through stimulation of phagocytosis.[32,33] 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[.34] ALV also has numerous anti-inflammatory agents like carboxypeptidase which inactivates bradykinin thereby reducing prostaglandin synthesis and inhibiting oxidation of arachidonic acid, which might decreases inflammation and relieves pain.

The response of the participants to the herbal product, as evaluated by questionnaire in the current study was good. In addition, no side effects have been noticed at the end of the study, which might add to its clinical usage as an adjunct to mechanical oral hygiene measures.

IV. Conclusion

On the basis of the results obtained, it can be concluded that ALV has a promising plaque inhibitory potential, although less than CHX. Further studies of longer duration in which antigingivitis effect can be also assessed along with evaluation of safety and microbiological parameters, are essential establish the true effectiveness of this mouthrinse.

References

[1]. Fine DH. Mouthrinses as adjuncts for plaque and gingivitis management: A status report for the American Journal of Dentistry. Am J Dent 1988; 1: 259–263.
[2]. De Vore L. The rinse cycle. RDH 2002; 93: 82–83.
[3]. Addy M. The use of antiseptics in periodontal therapy. Clinical Periodontology and Implant Dentistry, 4th edn. Copenhagen, Blackwell Munksgaard; 2003: 464– 493.
[4]. Sawai MA. Aloe Vera – A Miracle Herb. IJRID 2014 ; 4 Suppl 2: 1-7.
[5]. Vogler BK, Ernst E. Aloe vera: a systematic review of its clinical effectiveness. Br J Gen Pract 1999 Oct; 49:823-828.
[6]. Ndhlala AR, Amoo SO, Stafford GI, Finnie JF, Vand SJ. Antimicrobial, anti-inflammatory and mutagenic investigation of the South African tree aloe (Aloe barberae). J Ethnopharmacol 2009 Jul 30; 124 Suppl 3:404-408
[7]. Turesky S, Gilmore ND, Glickman I. Reduced plaque formation by the chloromethyl analogue of vitamin C. J Periodontol 1970; 41: 41–43.
[8]. Quigley GA, Hein JW. Comparative cleansing efficiency of manual and power brushing. J Am Dent Assoc 1962; 65: 26–29.
[9]. Saxer UP, Muhlemann H. Motivation und Aufklarung. Schweiz Monatsschr Zahnmed 1975 ; 85: 905–919.

[10]. Lorenz K, Bruhn G, Netuschil L, Heumann C, Hoffmann C. How to select study designs and parameters to investigate the effect of mouthrinses? Part I: Rationale and background. J Physiol Pharmacol 2009; 60 suppl 8:77-83
[11]. Lorenz K, Mayer D, Bruhn G, Noack B, Brecx M, Heumann C et al. Effect of N-chlorotaurinemouthrinses on plaque regrowth and vitality. Clin Oral Invest 2009; 13: 9–14.
[12]. Mandel ID. Chemotherapeutic agents for controlling plaque and gingivitis. J Clin Periodontol 1988 ; 15: 488–498.
[13]. Paraskevas S. Randomized controlled clinical trials on agents used for chemical plaque control. Int J Dent Hyg 2005; 3 suppl 4:162-78.
[14]. Moran J, Addy M, Kohut B, Hovliaras LA, Newcombe RG et al . Efficacy of mouthrinses in inhibiting the development of supragingival plaque over a 4-day period of no oral hygiene. J Periodontol 1994 ; 65: 904–907.
[15]. Singh A, Daing A, Dixit J. The effect of herbal, essential oil and chlorhexidine mouthrinse on de novo plaque formation. Int J Dent Hyg. 2013 Feb; 11 suppl 1:48-52.
[16]. Pizzo G, Compilato D, Di Liberto B, Pizzo I, Campisi G. Effects of two essential oil mouthrinses on 4-day supragingival plaque regrowth: a randomized cross-over study. Am J Dent. 2013 Jun; 26 suppl 3 :156-60.
[17]. Gupta RK, Gupta D, Bhaskar DJ, Yadav A, Obaid K, Mishra S. Preliminary antiplaque efficacy of aloe vera mouthwash on 4 day plaque e-growth model: randomized control trial. Ethiop J Health Sci. 2014 Apr; 24 suppl 2 :139-44.
[18]. Villalobos OJ, Salazar CR, Sánchez GR. Effect of a compound mouthwash Aloe vera in plaque and gingival inflammation. Acta Odontol Venez 2001;39 suppl 5 :16-24.
[19]. de Oliveira SM, Torres TC, Pereira SL. Effect of a dentifrice containing Aloe vera on plaque and gingivitis control. A double-blind clinical study in humans. J Appl Oral Sci. 2008; 16 suppl4 :293-6.
[20]. Chandrahas B, Jayakumar A, Naveen A, Butchibabu K, Reddy PK, Muralikrishna T.A randomized, double-blind clinical study to assess the antiplaque and antigingivitis efficacy of Aloe vera mouth rinse. J Indian Soc Periodontol 2012; 16 suppl 4: 543–548
[21]. Pradeep AR, Agarwal E, Naik SB. Clinical and microbiologic effects of commercially available dentifrice containing aloe vera: a randomized controlled clinical trial. J Periodontol 2012 Jun; 83(6):797-804.
[22]. Karim B, Bhaskar DJ, Agali C, Gupta D, Gupta RK, Jain A. Effect of Aloe vera mouthwash on periodontal health: triple blind randomized control trial. Oral Health Manag 2014 Mar; 13(1): 9-14
[23]. Simonson T, Ronstrom A, Rundegren J, Birkhed D. Rate of plaque formation--some clinical and biochemical characteristics of "heavy" and "light" plaque formers. Scand J Dent Res 1987; 95: 97-103.
[24]. Maclure M. The case-crossover design: a method for studying transient effects on the risk of acute events. Am J Epidemiol 1991 Jan15; 133 suppl 2:144-53.
[25]. Quirynen M ,Van Steenberghe D. Is early plaque growth rate constant withtime? Journal of Clinical Periodontology 1989 ; 16: 278–283.
[26]. Söder PÖ , Jin LJ , Söder B. Computerized planimetric method for clinical plaque measurement. Scand J Dent Res. 1993 Feb; 101 suppl 1:21-25.
[27]. Eaton KA, Kieser JB, Baker R. Assessment of plaque by image analysis. J Clin Periodontol 1985; 12 suppl 2:135– 140.
[28]. Renton-Harper P, Claydon N, Warren P, Newcombe RG,Addy M. Conversion of plaque-area measurements to plaque index scores: An assessment of variation and discriminatory power. J Clin Periodontol 1999 ; 26: 429-433.
[29]. Kambizi L, Afolayan AJ .Extracts from Aloeferox and Withania somnifera inhibit Candida albicans and Neisseria gonorrhoea. African J Biotech 2008; 7:12-15
[30]. Somboonwong J, Thanamittramanee S, Jariyapongskul A, Patumraj S. Therapeutic effects of Aloe vera on cutaneous microcirculation and wound healing in second degree burn model in rats. J Med Assoc Thai 2000; 83:417-425.
[31]. Korkina L, Suprun M, Petrova A, Mikhalchik E, Luci A, Deluca C. The protective and healing effects of natural antioxidant formulations based on ubiquinol and Aloe vera against dextran sulphate – induced ulceration colitis in rats. Biofactors 2003; 18: 255-264
[32]. Ferro VA, Bradbury F, Cameron P, Shakir E, Rahman S, Shimson WH.. In vitro susceptibilities of Shigella flexneri and Streptococcus pyogens to inner gel of Aloe barbadensis Miller. Antimicrob agents Chemother 2003; 47:1137-1139
[33]. Pugh N, Ross A, Elsohly MA, Pasco DS. Characterization of Aloeride, a new high-molecular weight polysaccharide from Aloe vera with potent immunostimulatory activity. J Agric Food Chem 2001; 49:1030-1034.
[34]. Lee Ss. Zhang W, Li Y. The antimicrobial potential of 14 natural herbal dentrifices: Results of an in vitro diffusion method study. J Am Dent Assoc 2004; 135:1133-41
[35]. Table 1: Mean values of plaque parameters (PI and PA) after four days of de novo plaque formation
[36].

	PI			PA (%)		
Group	Mean±SD	Min.	Max.	Mean±SD	Min.	Max.
CHX	2.84±0.14	2.59	3.07	38.16±2.50	34.32	41.56
ALV	3.48±0.16	3.26	3.71	48.20±2.25	45.20	51.42
PM	3.83±0.17	3.54	4.06	55.17±2.62	50.96	58.68
	F value=122.418 P value <0.001			F value=145.142 P value<0.001		

PI: Plaque index, PA : Plaque area ,CHX: Chlorhexidine mouthrinse , ALV: Aloe vera mouthrinse , PM: placebo mouthrinse

Table 2:Intergroup comparison of plaque parameters (PI and PA) of three mouthrinses on day four

	Dependent variables			
	PI (SE- 0.04558447)		PA (SE-0.00710107)	
Group	Mean difference	P-value	Mean difference	P-value
CHX vs ALV	-0.64261	<0.0001	-0.10040	<0.0001
CHX vs PM	-0.99467	<0.0001	-0.17018	<0.0001
ALV vs PM	-0.35207	<0.0001	-0.06978	<0.0001

PI: Plaque index, PA: Plaque area CHX: Chlorhexidine mouthrinse , ALV: Aloe vera mouthrinse , PM: placebo mouthrinse. The mean difference is significant at the
P< 0.05 level