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Antibacterial Efficacy of Aloe Vera Extract Mouth Wash versus Chlorhexidine in Pediatrics: An in Vivo Study

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ABSTRACT

Purpose: To assess and compare the antibacterial efficacy of Aloe vera (AV) extract solution and chlorhexidine (CHX) as mouth washes for children. **Material and methods:** Forty children of age range (5- 12) years were enrolled in this study. The participants were randomly divided into two equal groups; A& B (n= 20). Participants were asked to rinse with 10 ml of either 100% AV extract or 0.125% CHX mouthwashes (in group A & B respectively) for 4 days twice daily (after breakfast and lunch) for one minute and not to rinse with water thereafter. Saliva samples were collected at 0 (base line) (S1) and after 4 days use (S2). All collected saliva samples, were submitted to microbiology laboratory for total bacterial counting at both intervals for the two groups, the data were then collected, tabulated and statistically analyzed. **Results:** In both AV and CHX groups, the total bacterial count was decreased with a significant difference ($P \leq 0.05$) between the base line and after 4 days samples. In CHX group however, there was a significant decrease in total bacterial count compared to AV group. **Conclusion:** AV mouth wash has a comparable antibacterial effect to CHX mouth wash when used for children's oral health care.

KEYWORDS

Aloe vera, chlorhexidene, antibacterial mouth wash.

INTRODUCTION

With the progressive development in dental field, dental caries is still a problem all over the world. Inspite of being a multifactorial disease, no one can deny the important role of bacteria as the main etiologic factor [(1)]. Without bacteria, caries cannot develop or progress. Oral cavity is a shelter for 500- 1000 different types of bacteria besides fungi, protozoa and may be viruses. Full eradication of bacteria is practically unachievable, but, a decrease in the bacterial count can hinder the cariogenic process [(2)].

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Oral bacteria accumulate to form a complex dental biofilm. Dental biofilms are not always easily removed by mechanical hygiene measures. Causes may be technique difficulty for some persons as for children, handicapped and too much busy individuals [3]. Miller, in 1890, stated that antiseptics could kill or reduce the number and activity of bacteria. Antiseptics, therefore, can be used to disrupt the organized biofilm and destroy the bacterial cell [4]. Various chemical antiseptics are used. Mouth washes are examples that are generally preferred due to ease of use [5].

Synthetic mouth washes are often represented by chlorhexidine as the most popular one. It is a synthetic cationic bisguanide that is effective against both gram positive and gram negative bacteria. Staining of teeth and tongue and altered taste are limitations for its prolonged use. Scarce side effects, however, have brightened the need for utilizing natural antibacterial herbs as green tea and Aloe vera[6-8].

Aloe babadensis Mill (Aloe vera) herb contains a gel with strong antibacterial, antifungal and antiviral actions. Its major active components are; aloin, aloe- emodin, aloe mannan, ace mannan, aloride, naftoquinones, methyel chromones, flavonoids, saponin, sterols, aminoacids and vitamins. Aloe vera had proven efficacy in many oral uses as in managing recurrent ulcerations, lichen planus, candidiasis, extraction socket, root canal medication and in dentifrices [9-11].

Searching for a natural herb- based mouth wash that was effective and easy to use, will be a valuable issue for both parents and children. Therefore, this study was conducted to evaluate and compare the effect of Aloe Vera extract mouth wash to chlorhexidine on the total oral bacterial count in children.

MATERIAL AND METHODS

This study had been approved by the Ethical Committee, Faculty of Dental Medicine for Girls, Al- Azhar University, Cairo, Egypt. Also, informed consent forms were signed by the parents of the participants before conducting the research.

Preparation of mouth washes:

Aloe vera extract mouth wash was prepared by adding 0.1 g of pure organic AV inner gel powder (Indigo-herbs, Glastonbury, UK) to 100 ml of distilled water to obtain 100%[12] AV extract mouth wash.

Chlorhexidene mouth wash: 125mg/100ml= 0.125% CHX hydrochloride is commercially available; Hexitol (The Arab Drug Co. for pharmaceuticals & Chemical Industries, Cairo, A.R.E).

Case selection:

A total of forty Egyptian children within the age range of 5- 12 years of both sexes, from pediatric patients of the outdoor clinics of Faculty of Dental Medicine for Girls, Al- Azhar University, were included in this study. Inclusion criteria were: Medically-free children, no untreated carious lesions, absence of fixed or removable orthodontic appliances or prostheses, no history of recent antibiotic therapy at least two weeks prior to conduction of the study, no history of another antimicrobial mouth wash use at least several hours before the start of the study and no change of dietary habits and daily practices along the study interval [10].

Grouping of participants:

The participants were randomly divided into two equal groups; A& B (n= 20), according to whether participants were instructed to rinse with either 100% Aloe Vera extract or 0.125% chlorhexidine mouth washes (in group A & B respectively) for 4 days twice daily (after breakfast and lunch) for one minute and not to rinse with water thereafter.

Saliva sampling:

Saliva samples were taken at 0 (base line); S1 and after 4 days; S2 of twice daily use of the mouth washes under investigation, according to different

groups. Samples were collected at least 1 hour after meal & before tooth brushing [13]. At each assessment interval, the participant was asked to spit about (3 ml) in a labelled sterile container.

Microbiological analysis for total bacterial count:

All collected saliva samples, were immediately submitted to the Culture & Sensitivity Unit at Regional Center for Mycology & Biotechnology at Al- Azhar University. Each saliva sample was diluted (1: 100 and 1: 1000). For each dilution, 20 microliters of the sample were taken by micropipette from the sterile container. Diluted samples were then innoculated in plate count agar media (also known as Trypticase Glucose Agar, Standard Methods Agar). Each sample was cultured in triplicate. The plates were incubated at 37◦C for 24- 48 hours [14]. After the incubation period, colony forming units of each saliva sample were determined by using the number of colonies in a given dilution.

RESULTS

Statistical analysis

Colony forming units' values were presented as mean and standard deviation (SD) values. Data were explored for normality using Kolmogorov-Smirnov test of normality. The results of Kolmogorov-Smirnov test indicated that data were normally distributed (parametric data). Therefore, independent t test was used to compare both groups (AV & CHX), while paired t test was used for intragroup comparisons (base line and after 4 days).

The percent change in the number of colony forming units was calculated by the formula

$$\frac{\text{After 4 days value} - \text{base line value}}{\text{Base line value}} \times 100$$

The significance level was set at $p \leq 0.05$. Statistical analysis was performed with SPSS 18.0 (Statistical Package for Scientific Studies, SPSS, Inc., Chicago, IL, USA) for Windows.

In Aloe Vera extract (AV) group, the colony forming units (total bacterial count) significantly decreased after 4 days of use compared to base line. (p=0.00), (Table 1, Fig.1: a, b & Fig. 2))

In chlorhexidine (CHX) group, the colony forming units (total bacterial count) significantly decreased after 4 days use compared to base line. (p=0.00), (Table 2, Fig.1: c, d & Fig. 2).

Regarding both AV & CHX groups, at base line**,** there was no significant between both AV & CHX groups (p=0.075). After 4 days however, a higher mean value was recorded in AV group (2.52±0.56) in comparison to CHX group (1.54±0.14). Independent t test revealed that the difference between both groups was statistically significant (p=0.00), (Table 1).

Comparing the percent of change in total bacterial count revealed a greater percent decrease in colony forming units (total bacterial count) in CHX group (-82.79±1.84), in comparison to AV extract (-70.92±4.48). Independent t test revealed that the difference between both groups was statistically significant (p=0.00), (Table 2, Fig.3).

Table (1): *Descriptive statistics and comparison of Colony forming units (total bacterial count) (log 10) at base line & after 4 days in AV & CHX groups (paired t test).*

	AV		CHX	
	Base line	**After 4 days**	**Base line**	**After 4 days**
Mean	8.59	2.52	8.99	1.54
SD	0.80	0.56	0.25	0.14
Min	7.23	1.75	8.63	1.32
Max	9.86	3.40	9.38	1.76
t- value	11.8		13.5	
P- value	0.00*		0.00*	

*Significance level p≤0.05, *significant, ns=non-significant.*

Table (2): *Descriptive statistics and comparison of colony forming units (total bacterial count) (log 10) and percent of change (independent t test) between AV & CHX groups at base line & after 4 days.*

	Base line		After 4 days		Percent change	
Groups	**AV extract**	**CHX**	**AV extract**	**CHX**	**AV extract**	**CHX**
Mean	8.59	8.99	2.52	1.54	-70.92	-82.79
SD	0.80	0.25	0.56	0.14	4.48	1.84
Min	7.23	8.63	1.75	1.32	-77.28	-85.14
Max	9.86	9.38	3.40	1.76	-65.25	-80.02
t- value	1.84		6.57		9.49	
P- value	0.075ns		0.00*		0.00*	

*Significance level p≤0.05, *significant, ns=non-significant.*

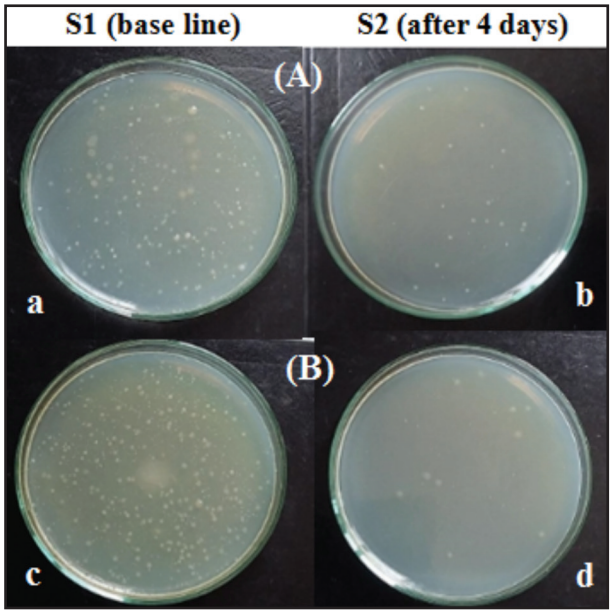

Figure (1): Plate count agar media showing colony forming units (total bacterial count) at base line; S1(a, c) and after 4 days S2; (b, d) for both AV (A) & CHX (B) groups respectively.

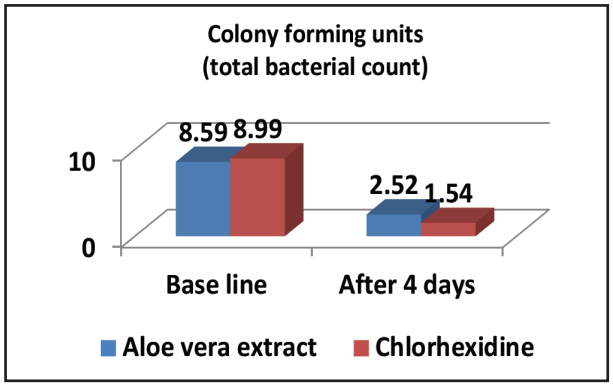

Figure (2): Bar chart showing mean colony forming units (total bacterial count) in AV & CHX groups.

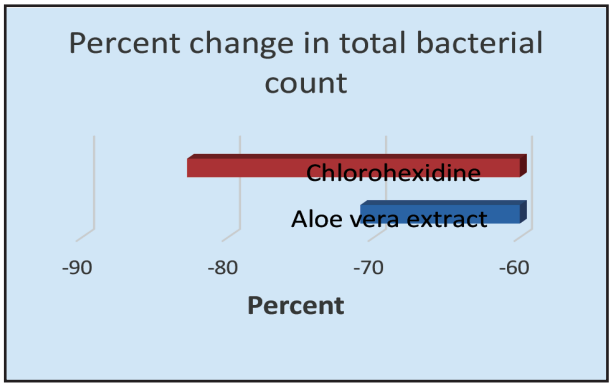

Figure (3): Bar chart showing mean percent change in colony forming units (total bacterial count) in AV & CHX groups.

DISCUSSION

For long decades CHX is the father of oral antiseptics. On prolonged use, however, staining of teeth and tongue and unpleasant taste may supervene [15]. With progressive trends in phytotherapeutics (plant extracts), natural herbs have come to be better substitutes [16]. Herbal essential oils are capable of killing bacteria on tooth surfaces; hindering biofilm and caries progression [17]. Phytotherapeutics, nowadays, have expanded uses in dentistry as antimicrobials, having less harms on the long run [18,19].

This study investigated the effect of 100% AV extract aqueous solution and compared it with 0.125% CHX mouth wash on total salivary bacterial

count. AV gel is composed of about 99.5% water while the active ingredients are about 0.5% only [20]. Accordingly, to get more concentration and value of the active ingredients, AV powder (latex) was utilized instead of the pure gel for mouth wash preparation. Samples were collected from saliva rather than plaque due to more constant bacterial count[21]. Sampling was done at least one hour after meal and before tooth brushing to escape possible fluctuations in microbial counts that occur throughout the day [13].

Results of this study indicated statistically significant bacterial activity of 100% AV mouth wash against oral pathogens after 4 days of twice daily use. Such efficacy was proven in several previous studies [22- 24]. AV is effective against both gram negative and gram positive species [25]. This is ascribed to about 26 bioactive constituents with antimicrobial properties; of which are; anthraquinones, dihydroxyanthraquinones, saponins, acemannan and aloe- emodin [26]. Such combination of active ingredients exerts different mechanisms of antibacterial activity being direct, as for aloin and aloe- emodin, via inhibiting bacterial protein synthesis, or indirect, as for acemannan acting by phagocytosis [11].

Regarding CHX group, a significant antibacterial activity was evident after 4 days of twice daily use. The current study therefore, emphasizes the potent antibacterial efficacy of CHX. CHX was recorded to be bacteriostatic at low concentrations, but bactericidal at high ones by coagulating bacterial cytoplasm [27].

In this study, the antibacterial efficacy of AV was far less than that of CHX. This could be attributed to the fact that the main active antibacterial ingredients of AV are anthraquinones; mainly aloin and aloe-emodin, which are phenolic compounds [28]. The antibacterial activity of AV is mainly affected by the location and quantity of hydroxyl groups in its phenolic active ingredients [29]. Saliva proline- rich proteins have high affinity for phenolic compounds via hydrogen bonding to their hydroxyl groups, forming saliva protein–polyphenol precipitates[30], thus suppressing the antibacterial activity of AV when used as mouth wash.

The lagging antibacterial efficacy of AV behind CHX, proven in the current study, gets along with another study [30], in which 2% CHX gel had totally suppressed E. faecalis; while the antibacterial behavior of AV and calcium hydroxide were only 78.9% and 64.3% respectively.Also, other studies [31,32] found CHX and propolis, were far more effective antibacterials compared to AV. The sitting study, notably, investigated the total bacterial count rather than an odd species. Different from these results were Vangipuram et al. [7] and Karim et al. [15] studies; both declared statistically non-significant difference between AV and CHX. Their studies, however, investigated the antiplaque efficacy and clinical effects on periodontitis, with no regards to the effects on bacterial counts differing in that way from the present study.

CONCLUSIONS

AV mouth wash seemed to have comparable antibacterial effects to CHX to be used in children's routine oral health care. Further in vivo studies of larger sample size and longer duration, investigating subfractions of AV active ingredients as odds, might prove more AV efficacy against oral pathogens.

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