Effect of Aloe Vera mouthwash on moderate gingivitis: Clinical and microbiological study

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Background and objectives: Gingivitis is an inflammatory process confined to the gingiva with no attachment or bone loss, Aloe Vera (AV) is an ancient medicinal plant that is widely known for its medicinal uses. This study was planned to assess the effect of AV as a mouthwash on clinical periodontal parameters; plaque index (PI), gingival index (GI) and bleeding index (BI) and to evaluate its antibacterial effect on bacterial plaque *Staphylococcus Aureus* and *Streptococcus Viridans*, comparing it with chlorhexidine (CHX) mouthwash.

Subjects and methods: Sixty patients with moderate gingivitis were randomly assigned to three groups. The subjects in the first group (A) used 0.12% CHX mouthwash, the second group (B) used 80% AV mouthwash and the third group (C) used 0.9% normal saline as placebo for 14 days. Clinical examinations to assess PI, GI and BI were done at days 0, 14 and 21 post treatment. The antibacterial activity of AV mouthwash was tested using Agar Well Diffusion method. Statistical Analysis: SPSS version 25. Mean ± (SD), normality test, ANOVA and T test.

Results: Significant reduction in the mean of PI, GI and BI at day 14 and 21 by using AV and CHX mouthwashes and statistically non-significant difference was observed between them. Means of the inhibition zone diameter against *Streptococcus Viridans* for CHX and AV were (15.64 ± 0.71 and 13.52 ± 1.02) respectively and against *Staphylococcus Aureus* were (23.33 ± 1.52) for CHX and (20 ±1) for AV.

Conclusions: The use of AV mouthwash results in improvement of periodontal status. It was also found that it has significant anti-bacterial and anti-inflammatory activity. Thus, it may be used as a treatment for plaque-induced gingivitis.

Key words: Aloe Vera, gingivitis, chlorhexidine, antibacterial activity.

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Introduction

Gingivitis is an inflammatory process confined to the gingiva with no attachment or alveolar bone loss,[1] it is one of the most popular oral diseases affecting more than 90% of the population, regardless of age, sex or race.[2] It is known that plaque is the primary etiological and initiating factor in the development of gingivitis when in contact with the gingival tissue, therefore, plaque control represents the cornerstone of a good oral hygiene practice.[3] A large number of commercial chemical plaque control agents are available, but have some deficiencies, chlorhexidine is considered as a gold standard,[4] but it cannot be prescribed for long periods of time as it may cause teeth staining, taste disturbance and in rare cases, painful desquamation of oral mucosa.[5] In addition to chlorhexidine, some essential oils have been widely used as oral rinses, however, the alcohol content and unfavorable taste are unacceptable to some

patients based on their religious beliefs.[4]

Aloe Vera is an ancient medicinal plant that has maintained its popularity over time. It is widely known for its medicinal uses in wound healing, as an analgesic, and for its anti-inflammatory [6] and antibacterial effects, it is also considered to have a good antiviral and antifungal properties.[7] Aloe Vera liquid has shown a wide range of anti-bacterial effect against both gram positive and gram negative bacteria, it was reported to effectively inhibit or significantly reduce the growth of *Staphylococcus Aureus, Streptococcus Mutans, Streptococcus Pyogenes, Pseudomonas Aeruginosa, Escherichia Coli, Helicobacter Pylori* and many others.[7]

The bactericidal activity of Aloe Vera against plaque bacteria is attributed to a number of pharmacologically active compounds including anthraquinones, aloin, aloe-emodin, aloetic acid, anthracine, aloe mannan, aloeride, antranol, acemannan, chrysophanic acid, resistanol, dihydroxy-anthraquinones and saponins.[8,9] Aloin and aloe-emodin have polyphenolic structures, which can inhibit protein synthesis by bacterial cells, thus explaining their anti-microbial activity. This characteristic may also explain the anti-inflammatory activity of Aloe Vera.[8,9]

Saponins, contains glycoside, a soapy substance that have both cleansing and anti-septic properties. It is noteworthy that some compounds like anthraquinones and saponins present in Aloe Vera gel have a direct antibacterial activity while some other components, such as acemannan, have been considered to show indirect bactericidal activity through stimulation of phagocytosis.[10]

Streptococcus Viridans (S. Viridans) group of bacteria can be considered as the backbone for dental plaque formation, as the plaque accumulation starts with pellicle formation then bacteria begins to attach to the outer surface of the pellicle, within the first two days in which no further cleaning is undertaken, the teeth surface is colonized predominantly by *Streptococcus Viridans*.[11] *Staphylococcus Aureus (S. Aureus)* is not generally considered to form part of the normal oral flora, and when isolated from the mouth, have been considered as transient colonizer. The role of intraoral *Staphylococcus Aureus* in oral diseases and infections is controversial, although some studies show its role in angular cheilitis, gingival disease and oral mucositis.[12] Therefore these two bacteria were selected for the study.

Although the medicinal use of Aloe Vera has been reported, not many literatures are available regarding its use in dentistry as a mouthwash. Hence the purpose of this study was to assess the effect of Aloe Vera as a mouthwash on clinical periodontal parameters (PI, GI and BI) in patients with moderate gingivitis at baseline (prior to treatment), after 14 and 21 days, and compare it with chlorhexidine mouthwash. Also to evaluate the antibacterial effect of Aloe Vera mouthwash on bacterial plaque *(Staphylococcus Aureus and Streptococcus Viridans)* by measuring the inhibition zone on agar using Agar Well Diffusion method at baseline before intra oral examination and comparing it to chlorhexidine mouthwash.

Subjects and methods

Setting of the Study. The present study was carried out in the Department of Periodontology at the College of Dentistry - Hawler Medical University, the biochemical and laboratory tests were made at Medya Diagnostic Center in Erbil city. Sixty patients aged from 20 – 35 years with moderate gingivitis who are systemically healthy with a minimum of twenty natural teeth available and with no supra-gingival plaque and calculus retentive areas such as partial denture or orthodontic appliances have participated in the study. Participants with probing pocket depth > 3 mm, patients receiving periodontal therapy three months prior to the study, pregnant or lactating women, smokers and patients with crowned teeth were excluded from the study.[8] The practical part was carried out during the period between 15th of February and 1st of June 2018.

Study Design. The patients were randomly assigned to three groups (20 patients each group). During the study period the subjects in the first group (group A) were using 0.12% Chlorhexidine mouthwash, whereas those in the second group (group B) were using 80% Aloe Vera

mouthwash and the third group (group C) were using 0.9% normal saline as placebo.[3,13]

In all the selected participants, clinical examination was performed to all the dentition to assess plaque index (PI),[14] Loe and Silness gingival index (GI),[14] and bleeding index according to Ainamo and Bay,[15] which is measured via gentle probing of the orifice of the gingival crevice. If bleeding occurred within ten seconds, a positive finding was recorded then the number of positive sites expressed as a percentage of the number of sites examined. All indices were calibrated by the examiner and the values were recorded using four point periodontal charting at baseline (day 0), and the subjects were recalled back to the clinic after 14 days and after 21 days for re-evaluation. The PI, GI and BI were re-evaluated in each recall.

Patients were advised to follow regular oral hygiene practices i.e., brushing and flossing twice daily, and to rinse with 10 ml of the solution twice a day for a duration of one minute, once every morning, and once before bedtime and instructed to use the mouthwash for 14 days. Ingestion of liquids or solids was permitted only after 30 minutes of rinsing.[8]

Dark glass bottles of identical appearance with a volume of 300 ml of solution were dispensed to each participant, a measure of 10 ml scale to ensure dosage accuracy was dispensed for the patients, a low ingredients tooth paste was provided for the patients during the study period in order to minimize its effects on the study results, identical soft tooth brushes were provided for the patients.

Before the clinical examination, the teeth were isolated using a cheek retractor and dried, then a supra-gingival plaque sample was collected from the facial surface of anterior teeth and premolars of each patient using a curette and stored in 2 ml of normal saline thereafter transferred to the laboratory to be cultured within 30 minutes.

Preparation of Aloe Vera Mouthwash. Mature, healthy and fresh leaves of AV were washed using running tap water and rinsed with sterile distilled water, and then dissected longitudinally, the colorless parenchymatous tissue (aloe gel) without the fibers was scraped out using a sterile knife.[16] AV gel was transformed to a liquid form using a blender, aloe juice (80% Aloe Vera 20% normal saline) was prepared. AV mouthwash prepared by adding 98% aloe juice, 0.2% sodium benzoate crystal as a preservative and 0.001% lemon-lime flavor, these measurements were done using a sensitive scale.[13] The pH of the prepared mouthwash was measured to be equal to 7.3 using a pH meter.

For the microbiological study 10 ml of the prepared mouthwash was mixed with 10 ml of 2% Dimethyl Sulfoxide (DMSO) and kept at 4°C. DMSO was used as a solvent which has no antimicrobial effect of its own.[7] It is an important polar aprotic solvent that dissolves both polar and nonpolar compounds and is miscible in a wide range of organic solvents as well as water. It is extensively used as an extractant in biochemistry.[17]

Microbiological Laboratory Procedures. Supra-gingival plaque samples were collected from the patients and transferred to the lab in sterile tubes containing 2 ml of normal saline and cultured on SBA blood agar immediately at the laboratory, then the petri plates were incubated aerobically for 24 hours. The incubated petri plates were removed from the incubator after 24 hours, a mixed population of bacterial and fungal growth was found. The selected microorganisms (*S. Aureus and S. Viridans*) were identified and isolated then the anti-microbial activity was carried out by Agar Well Diffusion method against the selected microorganisms.[17]

Identification and Isolation of the bacteria. Hemolytic activity of the bacteria was observed on the blood agar, alpha hemolysis was noticed with *S. Viridans* while beta hemolysis was seen with *S. Aureus* growth. Both Streptococci and Staphylococci are gram positive cocci, therefore gram stain was performed to identify them from other gram negative cocci. The Streptococci are non-motile, often occur as chains or pairs,[18] and Staphylococci tend to be arranged in clusters.[19] Optochin discs were used to distinguish *S. Viridans* from other alpha hemolytic streptococci such as *S. Pneumonia*, and biochemical identification tests including the catalase test were used to

differentiate the Streptococci species that result in a negative catalase test from Staphylococci being catalase-positive. However, for the identification of *Staphylococcus Aureus*, the coagulase test was used which is positive for *Staphylococcus Aureus* distinguishing it from other Staphylococcus species, novobiocin was used to distinguish *Staphylococcus saprophyticus*, and mannitol fermentation positive to distinguish *Staphylococcus epidermidis*.[19] The streak -plate procedure was used to isolate pure cultures of bacteria, or colonies, from mixed populations by simple mechanical separation. With this procedure, a mixture of cells is spread over the surface of a semi-solid, agar-based nutrient medium in a Petri dish such that fewer and fewer bacterial cells are deposited at widely separated points on the surface of the medium, following incubation, develop into colonies and this procedure was repeated until pure colonies of the selected organisms were obtained.[20]

Agar Well Diffusion Method. The anti-bacterial activity of AV mouthwash was tested using Agar Well Diffusion technique by the evaluation of inhibition zone. A bacterial inoculum was made by sub-culturing the selected bacteria culture into normal saline and the optical density that matched to McFarland standard of 0.5. Subsequently, wells of 6 mm diameter cut on sterile SBA plates and the agar plate surface was inoculated by spreading a volume of the microbial inoculum over the entire agar surface. About 0.1 ml (100µl) of the AV mouthwash was poured into one of the wells, CHX as a positive control was filled into another one and normal saline was filled into the last one as a negative control, then incubated at 37°C ± 0.2°C. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested. Petri-plates were observed for inhibition zones. Antibacterial activity in terms of zones of inhibition (mm) was recorded after 24 hours of incubation, and the diameters were measured using vernier calipers.[9,17]

Statistical Analysis. The collected data were analyzed using computer program software SPSS (Statistical Package for Social Sciences) version 25 by IBM / USA. Descriptive statistics which include; means ± standard deviations (SD) and tables. Normality test was done to decide parametric or non-parametric test to be use. Inferential statistics included; F-test (One-way ANOVA) used to compare clinical variables (PI, GI and BI) at different time intervals and t-test was used to compare the mean of inhibition zone between AV and CHX.

Results were considered non-significant if $p \geq 0.05$, significant, if $p \leq 0.05$ and highly significant if $p \leq 0.01$.

Results

The total number of samples consisted of 60 patients with moderate gingivitis participated in the study out of which (62%, N = 37) were females and (38%, N = 23) were males. The mean age of the study participants was (25.53 ± 2.97) years.

The *S. Viridans* was isolated from 100% of the cultured study samples N = 60. Table 1 shows that; its sensitivity to AV mouth-wash, was significantly lower than the CHX as the mean of the inhibition zone diameter for CHX and AV was (15.64 ± 0.718 and 13.52 ± 1.023) respectively at P=0.00.

S. Aureus was positive only in 5% of the study samples and significantly more sensitive to CHX mouthwash than AV with mean inhibition zone of (23.33 ± 1.528) for CHX and (20 ± 1) for AV. This result was statistically significant at P=0.034 as shown in table 1.

Table 1: Comparison of the means of inhibition zones against *Streptococcus Viridans* and Staphylococcus Aureus when treated with Chlorhexidine and Aloe Vera mouthwashes.

Bacteria	Group	Mean	±SD	t-test	P value	Significance
S. Viridans	CHX	15.64	0.718	11.219	0.000	HS
	AV	13.52	1.023			
S. Aureus	CHX	23.33	1.528	3.162	0.034	S
	AV	20.00	1.000			

Table 2 shows the comparison of the mean of PI, GI and BI among the three study groups using the one-way ANOVA with post hoc test; Duncan multiple range test among the three groups at day 0, day 14, and day 21 post treatment. There is suggestion of a reduction in PI, GI and BI in all groups at day 14 post treatment, it also shows a statistically significant reduction in the PI, GI and BI at day 14 and 21 after treatment at $p \leq 0.05$. There is highly statistical significant reduction in the mean of GI at day 14 between group A and C and also between group B and C at $P=0.000$. However, the difference is not significant between group A and B. The reduction of the mean of BI was also significant among all of the study groups at day 14 and 21 post treatment.

Table 2: Comparison of the mean of plaque index, gingival index and bleeding index of the three study groups at different time intervals.

Parameters	Time (days)	Group	N	Mean	±SD	F-test	P value	Significance
PI	0	A	20	1.86$^{(b)*}$	0.301	8.216	0.001	HS
		B	20	1.80$^{(b)}$	0.399			
		C	20	1.50$^{(a)}$	0.181			
	14	A	20	1.09$^{(a)}$	0.386	5.472	0.007	HS
		B	20	1.03$^{(a)}$	0.413			
		C	20	1.36$^{(b)}$	0.169			
	21	A	20	1.25$^{(b)}$	0.316	8.653	0.001	HS
		B	20	0.96$^{(a)}$	0.336			
		C	20	1.30$^{(b)}$	0.125			
GI	0	A	20	1.49$^{(a)}$	0.147	0.242	0.786	NS
		B	20	1.52$^{(a)}$	0.151			
		C	20	1.51$^{(a)}$	0.031			
	14	A	20	1.21$^{(a)}$	0.167	40.956	<0.001	HS
		B	20	1.12$^{(a)}$	0.099			
		C	20	1.55$^{(b)}$	0.189			
	21	A	20	1.23$^{(a)}$	0.150	21.890	<0.001	HS
		B	20	1.15$^{(a)}$	0.113			
		C	20	1.46$^{(b)}$	0.185			
BI	0	A	20	50.15$^{(a)}$	14.705	0.182	0.834	NS
		B	20	51.25$^{(a)}$	11.153			
		C	20	49.15$^{(a)}$	4.891			
	14	A	20	20.45$^{(b)}$	14.087	61.726	<0.001	HS
		B	20	14.15$^{(a)}$	8.628			
		C	20	46.60$^{(c)}$	3.858			
	21	A	20	21.15$^{(b)}$	13.936	60.394	<0.001	HS
		B	20	13.90$^{(a)}$	9.602			
		C	20	47.00$^{(c)}$	3.783			

Discussion

Although inflammation is a necessary defensive mechanism, it can lead to host tissue destruction when the response is exaggerated.[6] Thus, recent treatments must aim at regulating excessive inflammation. A

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