

Comparison of Efficacy of Herbal Solutions in Chronic Gingivitis Patients- A Microbiological Study

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Abstract: The common etiology for initiation of gingival or periodontal disease is plaque accumulation. Plaque induced gingivitis is due to interaction between host inflammatory cells and microorganisms present in dental plaque. The present study includes the aloe vera and ocimum disinfectants as oral hygiene aids to reduce plaque formation and to know their efficacy over chlorhexidine mouthwash. Supragingival plaque samples were collected from patients between the age groups 18-55yrs with chronic generalized gingivitis (CGG). 15 plaque samples were collected and transferred to anaerobic culture media for culturing *Prevotella intermedia* species, then samples were immersed in disinfectants randomly which are divided into 3 groups: Group I: aloe vera juice, Group II: ocimum juice, Group III: Chlorhexidine and assessed for colony forming units with digital colony counter. The results showed that the bacterial count of *P. intermedia* reduced after rinsing the plaque sample with the disinfectants. All the three groups were statistically significant before and after treatment. The results of the study concluded that ocimum, aloe vera showed significant reduction in bacterial count in plaque samples but not superior to chlorhexidine.

Keywords: plaque, mouthwash, *Prevotella intermedia*

INTRODUCTION

Periodontal disease includes gingivitis, periodontitis, Necrotizing ulcerative disease, abscesses of periodontium, endo-perio lesions, development or acquired deformities and conditions, peri-implantitis [1]. Among these the most prevalent periodontal disease worldwide is chronic gingivitis. The cause of gingivitis may be plaque induced, drug induced, gingival disease modified by systemic factors, malnutrition or due to viral or fungal origin [2]. The common etiology for initiation of gingival or periodontal disease is plaque accumulation.

Plaque induced gingivitis is due to interaction between host inflammatory cells and microorganisms present in dental plaque. The microbiota of dental plaque induced gingivitis includes gram positive and gram negative species, as well as facultative and anaerobic microorganisms. Predominant gram positive species include *S.mitis*, *S.sanguis*, *S. Oralis*, *A.viscous*, *A.naelsundi*. The gram negative species predominantly include *P.intermedia*, *F.nucleatum*, *Heamophilus*, *Capnocytophaga*, *Campylobacter species* [3].

The mechanical means for plaque control are tooth brushing and interdental cleaning subgingival irrigation, chemical methods of plaque control is by using chlorhexidine, essential oils and various other synthetic chemical agents.

Chemical plaque controls are used as adjunct as they have the ability to inhibit growth, metabolism and colonization of bacteria. However, they have their own side effects. Chlorhexidine is the gold standard synthetic chemical agent for plaque control [4], but it

cannot be used for longer duration of time due its side effects. Thus to prevent the side effects caused by synthetic agents, use of natural agents (herbals) have come into existence.

The herbal products like *Aloe vera*, *Azadirachta indica*, *piper betel*, *Ocimum sanctum* possess antibacterial, ulcer healing, antiplaque and antihalitosis properties [5]. The present study includes the aloe vera and ocimum disinfectants as oral hygiene aids to reduce plaque formation and to know their efficacy over chlorhexidine mouthwash.

MATERIALS AND METHODS

Plaque sampling

Supragingival plaque samples were collected from patients with chronic generalized plaque-induced gingivitis visiting the Department of Periodontology, Meghna Institute of Dental Sciences, Nizamabad. Patients between the age groups 18-55yrs with Chronic Generalized Gingivitis (CGG) were included in the study. Factors as exclusion criteria were medically

compromised patients, any long term medications and patients who had used any type of antibacterial mouthwash in 4 weeks of commencement.

Samples of supragingival plaque (approximately 1 mg) were collected with a sterile explorer from the buccal and lingual molar surfaces of the mouth. It was then placed in transport media (thioglycate medium) until carried to the laboratory for microbial investigation. 100% pure Aloe vera juice (Natura Biotechnol pvt Ltd, India) 100% pure ocimum (Pancha Tulsi) Deltas Pharma, India, were used in the study.

Microbial study

The present in vitro study was conducted at Averin Biotech Private Limited, Hyderabad, and Telangana State, India. Plaque samples were collected from CGG patients. *Prevotella intermedia* (ATCC 25611) species were anaerobically cultured using blood agar as culture media and were incubated at 37°C for 3 days. They were later assessed for colony forming units using a digital colony counter.

The samples were then immersed in disinfectants for 1 minute and were again cultured for *P.intermedia* anaerobically for 72 hrs and assessed for colony forming units with digital colony counter. Later comparison of CFU/ml (Colony Forming Unit per ml) of *Prevotella intermedia* before and after treating with disinfectant and comparison of CFU/ml *P.intermedia* between three disinfectants was done using statistical analysis.

The 3 different groups used were

Group I: plaque samples disinfected with aloe vera juice and *Prevotella intermedia* count was done.

Group II: plaque samples disinfected with ocimum juice and *Prevotella intermedia* count was done

Group III: plaque samples disinfected with Chlorhexidine mouthwash and *Prevotella intermedia* count was done

STATISTICAL ANALYSIS

The values obtained were statistically analyzed using computer software Statistical Package for Social Sciences (SPSS) version 17.0. Paired t test was used to know the mean CFU/ml before and after treatment with disinfectant. Comparison of mean change in CFU per ml between treatments was done using ANOVA. P-value was set at <0.001

RESULTS

Table 1 represents Colony Forming Units of *P.intermedia* of 15 plaque samples before treatment and after treatment with various disinfectants. Pre-treatment & post treatment comparison was done using Paired t-test where there was a statistical significant difference (p-value <0.001) (Table 2,3,4] before and after treatment using disinfectants. Table 6 represents change in CFU/ml in aloe vera, ocimum and chlorhexidine was 416.07±61.1, 658.2±51.04 and 796±52.80963 respectively. Indicating that there was a statistically significant difference between the test groups in the mean colony forming units.

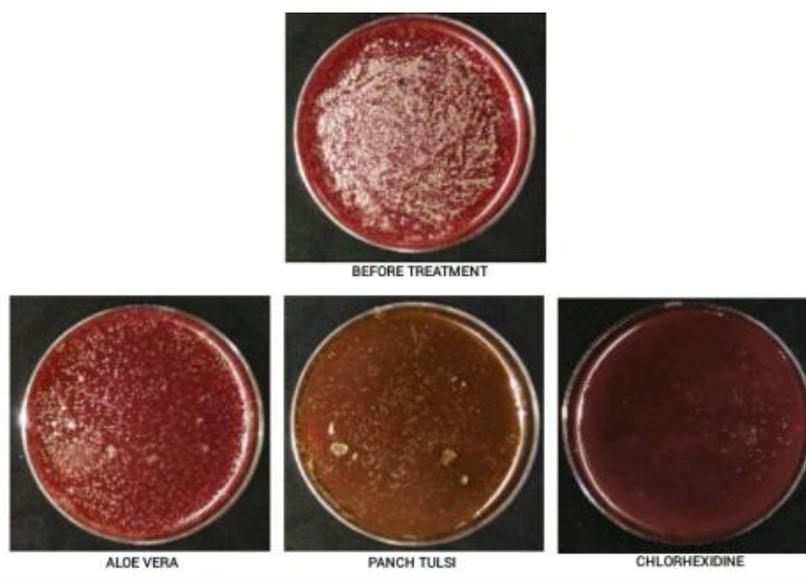


Fig-1: colony forming units of *P.intermedia* before treatment and after treatment with various disinfectants

Table-1: Colony Forming Units of P. intermedia of 15 plaque samples before treatment and after treatment with various disinfectants

S.No	Sample	CFU per ml before treatment	Group		
			CFU per ml after Aloe vera treatment	CFU per ml after ocimum treatment	CFU per ml after Chlorhexidine treatment
1	1	983 x 10 ² CFU/ml	416 x 10 ² CFU/ml	263 x 10 ² CFU/ml	121 x 10 ² CFU/ml
2	2	968 x 10 ² CFU/ml	479 x 10 ² CFU/ml	216 x 10 ² CFU/ml	67 x 10 ² CFU/ml
3	3	891 x 10 ² CFU/ml	481 x 10 ² CFU/ml	229 x 10 ² CFU/ml	93 x 10 ² CFU/ml
4	4	887 x 10 ² CFU/ml	523 x 10 ² CFU/ml	207 x 10 ² CFU/ml	119 x 10 ² CFU/ml
5	5	915 x 10 ² CFU/ml	562 x 10 ² CFU/ml	215 x 10 ² CFU/ml	101 x 10 ² CFU/ml
6	6	852 x 10 ² CFU/ml	485 x 10 ² CFU/ml	234 x 10 ² CFU/ml	127 x 10 ² CFU/ml
7	7	932 x 10 ² CFU/ml	534 x 10 ² CFU/ml	257 x 10 ² CFU/ml	68 x 10 ² CFU/ml
8	8	920 x 10 ² CFU/ml	463 x 10 ² CFU/ml	213x 10 ² CFU/ml	110 x 10 ² CFU/ml
9	9	843 x 10 ² CFU/ml	515 x 10 ² CFU/ml	268 x 10 ² CFU/ml	98 x 10 ² CFU/ml
10	10	860 x 10 ² CFU/ml	415 x 10 ² CFU/ml	232 x 10 ² CFU/ml	116 x 10 ² CFU/ml
11	11	842 x 10 ² CFU/ml	437 x 10 ² CFU/ml	212x 10 ² CFU/ml	102 x 10 ² CFU/ml
12	12	917 x 10 ² CFU/ml	493 x 10 ² CFU/ml	234 x 10 ² CFU/ml	89 x 10 ² CFU/ml
13	13	865 x 10 ² CFU/ml	412 x 10 ² CFU/ml	286x 10 ² CFU/ml	107 x 10 ² CFU/ml
14	14	849 x 10 ² CFU/ml	487 x 10 ² CFU/ml	232 x 10 ² CFU/ml	87 x 10 ² CFU/ml
15	15	935 x 10 ² CFU/ml	516 x 10 ² CFU/ml	288 x 10 ² CFU/ml	114 x 10 ² CFU/ml

CFU/ml- colony forming units per ml

Table-2: Comparison of mean CFU per ml aloevera before and after treatment

Treatment	CFU per ml	95% CI	Difference	95% CI	p-value
Pre Treatment	897.27±45.64	(871.99, 922.54)	416.07±61.13	(382.22, 449.92)	<0.001
Aloevera	481.2±45.76	(455.86, 506.54)			

Table-3: Comparison of mean CFU per ml ocimum before and after treatment

Treatment	CFU per ml	95% CI	Difference	95% CI	p-value
Pre Treatment	897.27±45.64	(871.99, 922.54)	658.2±51.04	(629.93, 686.47)	<0.001
Ocimum	239.07±26.9	(224.17, 253.97)			

Table-4: Comparison of mean CFU per ml chlorhexidine before and after treatment

Treatment	CFU per ml	95% CI	Difference	95% CI	p-value
Pre Treatment	897.27±45.64	(871.99, 922.54)	796±52.80	(766.755, 825.245)	<0.001
Chlorhexidine	101.27±18.07	(91.26, 111.27)			

Table-5:§Pre-treatment & post treatment comparison (P-value <0.001, Paired t-test);

Group	CFU per ml	Change in CFU per ml	p-value [¥]
Pre Treatment	897.27±45.64		<0.001
Aloevera [§]	481.2±45.76	416.07±61.13	
Ocimum [§]	239.07±26.9	658.2±51.04	
Chlorhexidine [§]	101.27±18.07	796±52.80963	

[¥]Comparison of mean change in CFU per ml between treatments (ANOVA)

[§] indicates statistical significant difference among the groups

Table-6: Comparison of mean change in CFU per ml between treatments

Treatment	Change in CFU per ml after treatment	p-value
Aloevera	416.07±61.13	<0.001
Ocimum	658.2±51.04	
Chlorhexidine	796±52.80963	

DISCUSSION

Gingival and periodontal disease can be controlled by various methods like mechanical brushing, flossing and chemical which include usage of mouthwashes [6]. Various synthetic mouthwashes have been introduced where chlorhexidine has been considered as gold standard. Chlorhexidine is an antimicrobial agent. It is a cationic bisbiguanide with broad antibacterial activity [7].

Loe & Schiott showed that rinsing with 10 ml of 0.2% w/v chlorhexidine gluconate mouthwash for 1 minute twice daily completely prevented the formation of plaque and gingivitis [8]. Chlorhexidine's superior antiplaque effect can be explained in terms of its superior degree of persistence at the tooth surface[4], but it has certain side effects like staining tooth surfaces, altered taste sensation etc [9]. Considering the side effects, cost effective alternative natural mouthwashes have been developed, but the studies comparing the efficacy of synthetic mouthwashes to natural mouthwashes is limited.

Aloe vera has anti-plaque action [10]. It acts effectively against a range of bacteria particularly against *Streptococcus mutans*. Vitamin C, hyaluronic acid and dermatan sulfate contents present in aloe vera are involved in collagen synthesis, and hence, provide relief in swelling and bleeding gums[11].

Agarwal *et al.* Manasa *et al.* has observed that ocimum acts effectively against *Streptococcus mutans* and *P.intermedia* respectively at various concentrations demonstrating its antibacterial and antiplaque action[12].

The present study is to compare the efficacy of aloe vera and ocimum mouthwash with chlorhexidine as it is considered as gold standard. The study compares the efficacy of the disinfectants on *Prevotella intermedia* as it is the common gram negative rod responsible for gingivitis. According to our knowledge, there are no studies comparing the efficacy of herbal mouthwashes and synthetic mouthwash against periodontal pathogen *P.intermedia*.

The results showed that the bacterial count of *P. intermedia* reduced after rinsing the plaque sample with the disinfectants. All the three groups were statistically significant before and after treatment. The present study showed that chlorhexidine showed better disinfection than aloe vera and ocimum disinfectant. The antimicrobial action of chlorhexidine is by binding to microbial cell membranes and damaging the surface structure, Chlorhexidine's superior antiplaque effect can be explained in terms of its superior degree of persistence at the tooth surface[4].

The presence of secondary metabolites such as steroid, triterpenoid, flavonoid, phenol, tannin, alkaloid, saponin and acid in the Aloe vera and ocimum would be responsible for the antibacterial activity [13].

The study showed that ocimum had a better disinfectant action than aloe vera. The difference in the antimicrobial efficacy of the plant extracts is suggested to be depended on the variation in their phytochemicals.^[14] Further studies are required to evaluate the disinfection action against other microbes and future research regarding the exact mechanism of disinfection action of the herbal solutions is required.

CONCLUSION

Among the experimental groups, chlorhexidine exhibited least number of colonies when compared to other disinfecting solutions. Ocimum was found to be the second most effective disinfecting solution while Aloe vera Juice was least effective among the solutions tested. Although synthetic mouthwash showed better efficacy than herbal mouthwash, there is a statistical significant difference in bacterial count with the usage of herbal disinfectants (comparing before and after treatment). Thus considering socioeconomic factors, side effects of chlorhexidine and/or preference of the population towards natural products presently tested herbal mouthwashes may be considered as a good alternative.

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