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EFFECT OF CINNAMON EXTRACT ON GINGIVAL HEALTH: A CLINICO-MICROBIOLOGICAL STUDY

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ABSTRACT

Background: Plaque being the main etiological factor in gingivitis, it is mandatory to remove plaque from hard and soft tissue surface using mechanical method and chemical method. Cinnamon is a commonly used spice by different cultures around the world for several centuries. Cinnamon extract may act as a good oral hygiene product because of its antimicrobial and anti-inflammatory activity. This study assess the efficacy of cinnamon as mouthwash in treatment of gingivitis. **Materials and Methods:** An in vitro analysis was done to determine the MIC of cinnamon tested on *S. gordonii* and *S. oralis* which was found to be 100mg/ml (10%) concentration and used further for the *in vivo* study. In this randomized controlled clinical trial, 30 subjects were randomly assigned to two groups with 15 subjects in each group, Group A- Scaling + 10% cinnamon mouthwash and Group B- Scaling + 0.2% Chlorhexidine mouthwash. Clinical parameters like Plaque index (PI), Gingival index (GI), Sulcus Bleeding Index (SBI) and Microbiological parameter like Gram's staining for quantitative assessment of Gram positive and Gram negative organisms were assessed at baseline and 1 month. **Results:** On intragroup comparison of clinical parameters, both the groups showed a statistically significant difference from baseline to one month with $p<0.001$. Intergroup comparison was statistically non-significant for clinical parameters. The microbiological analysis also was found to be statistically significant difference in gram staining in both the groups at 1 month ($p<0.001$). **Conclusion:** 10%

cinnamon mouthwash is almost equally effective as 0.2% chlorhexidine in treating chronic generalised gingivitis.

KEYWORDS: Gingivitis, Cinnamon Mouthwash, MIC, PI, GI, SBI.

INTRODUCTION

The oral health of a person speaks volumes about the overall health of a person. Recently, more importance has been laid on the potential of oral microorganism and oral inflammation having role in the progression of various systemic diseases. All over the world most reliable and accepted method of oral hygiene maintenance is mechanical methods of tooth cleaning. Adjunct to mechanical therapy for decreasing plaque formation and maintaining oral hygiene have been constantly on research.[1]

Gingivitis is the initial stage of periodontal disease that occurs primarily due to the plaque microorganisms.[2] Plaque being the main etiological factor in gingivitis, it is mandatory to remove plaque from hard and soft tissue surfaces using mechanical method and chemical method. Plaque is the main etiological factor periodontal inflammation, with gingivitis being the early manifestation of this process. With appropriate intervention, this process can be reversed and the periodontium returned to a state of health.

Tough chlorhexidine is considered as the gold standard for chemical plaque control but there are a few disadvantages associated with the long term use like altered taste sensation and staining. Some oral bacteria also develop resistance to the antibacterial activity of chlorhexidine[3]. These adverse effects of chlorhexidine directed towards the development of herbal oral hygiene products. Hence there is a requirement for a long term, home based remedy which should be economical.

Cinnamon is a commonly used spice by different cultures around the world for several centuries. Cinnamon *(Cinnamomum zeylanicum)* is a member of the Lauraceae family. It is one of the main herbs used extensively for treatment of several conditions. The bark of the cinnamon tree contains an essential oil called cinnamonaldehyde, which give cinnamon its characteristic flavour and aroma. From historical time, cinnamon has been used as a medicine for colds, flatulence, nausea and diarrhoea by improving energy, vitality, and circulation[4, 5]. Cinnamon is a coagulant and prevents bleeding[6]. The essential oil of cinnamon also acts as an

antioxidant. Many studies have found that cinnamon has antibacterial, anti-inflammatory and antifungal properties.[7, 8]

Cinnamon extract may act as a good oral hygiene product because of its antimicrobial and anti-inflammatory activity. Since, there are fewer studies regarding the efficacy of cinnamon as mouthwash in treatment of gingivitis, this study is carried out to assess the efficacy of cinnamon as mouthwash in treatment of gingivitis.

MATERIALS AND METHODOLOGY

A randomized clinical and microbiological study was conducted among the out patients visiting the Department of Periodontics, Sri Hasanamba Dental College and Hospital, Hassan. Total of 30 subjects who fulfil the inclusion and exclusion criteria were selected for the study (Figure 1). Randomization was done using chit method. The study design was approved by the Institutional Ethics Committee of Sri Hasanamba Dental College and Hospital, Hassan, Karnataka.

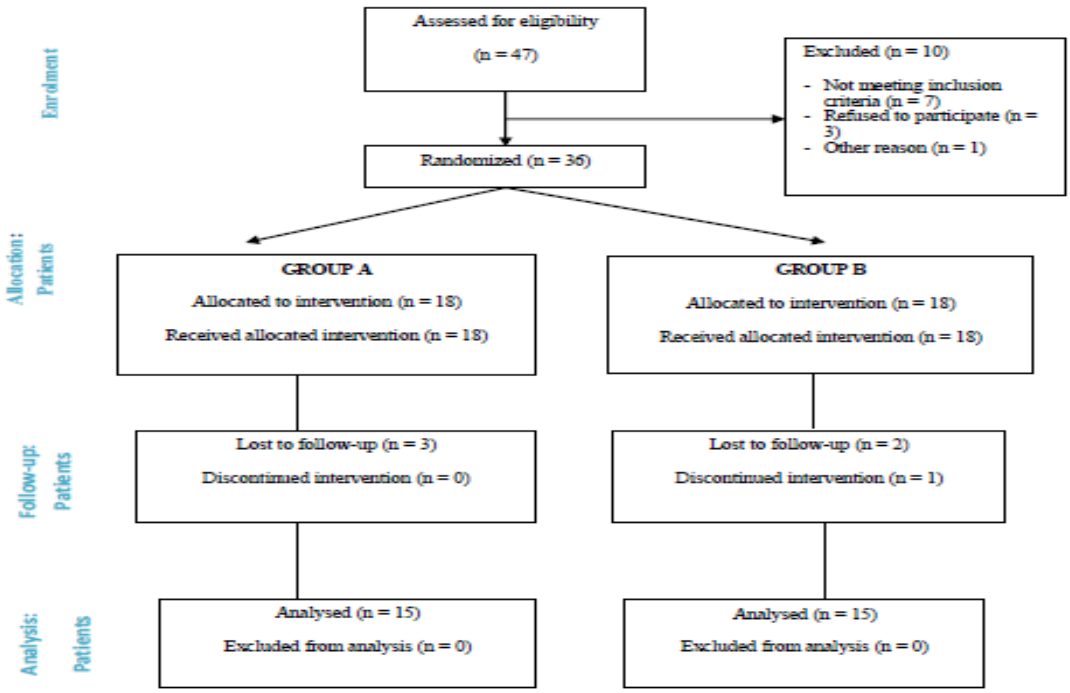

Figure 1: Consort chart.

Inclusion criteria

Subjects between 18-45 years of age and had clinical signs of gingivitis were included in the study. Those that have not undergone dental treatment for past 6 months and had full mouth bleeding score ≥ 1 were included. Subjects who had not taken any antibiotic or anti-

inflammatory drug therapy and not used any mouthwash in the preceding six months were included in the study.

Exclusion criteria

Subjects with any systemic diseases/conditions and on any medication which can affect periodontium were excluded. Participants allergic to cinnamon/other food spices were not included. Smokers, pregnancy and lactating mothers were excluded from the study.

In-vitro analysis

The antimicrobial effect of *Cinnamomum zeylanicum* on primary colonisers such as *Streptococcus oralis* and *Streptococcus gordonii* was analysed, by determining the minimum inhibitory concentration of the drug. The sample solutions of 5%, 10% and 15% were prepared for testing the MIC of the drug based on an in vitro study.

Bacterial sample preparation

S. oralis and S. gordonii strains preserved at Dextrose Technologies lab, Bangalore were sub-cultured 24 hours prior to the study.

Antibacterial activity assay

S. oralis and S. gordonii were grown in Brain heart infusion (BHI) liquid culture medium at 37 degree Celsius for 24 hours anti-bacterial activity was evaluated by well diffusion technique. After autoclaving the plates were allowed to dry and 6mm wells were punctured on the surface of agar plate. The agar plates were seeded with 100µl of the inoculums and spread evenly over the plate with sterile glass spreader. 200µl of each sample (5%, 10%, 15%) were added to separate wells in the culture plates and incubated at 37 degree Celsius for 24 hours. After 24 hours of incubation diameter of the zone of inhibition was measured to nearest millimetre. Cinnamon extract has showed a notable zone of inhibition in the culture plates with all the three concentration tested. The antibacterial activity is concentration dependent as highest inhibitory zone was observed with 15% sample of the cinnamon extract (Figure 4).

Based on the experimental results the antimicrobial activities of the samples were found to be effective at a concentration of 10% and above. Hence, 10% cinnamon was used for preparing the final mouthwash.

Method of preparation of cinnamon mouthwash

Cinnamon mouthwash was prepared at the Department of Pharmaceuticals in Sri Adichunchanagiri College of Pharmacy, B.G.Nagara, Bellur, Karnataka.

Preparation of cinnamon powder

The bark of cinnamon were collected from departmental store in Hassan, identified and authenticated by a taxonomist. The barks were thoroughly washed using water treated with reverse osmosis and shade dried over a period of 2-3 weeks at room temperature. The barks were hand crushed to obtain small pieces and prepared into a fine powder using a suitable armamentarium and was stored in airtight moist controlled containers and stored at room temperature.

Cinnamon mouthwash preparation

In this method, herbal mouthwash (10% w/w) was prepared using cinnamon powder. Firstly, 10 grams of cinnamon powder was weighed and transferred into 100 ml sterile beaker. Then, 10 ml of ethanol was added to the powder and mouth of beaker was tightly closed using aluminium foil and kept aside for one day after which it was filtered. In another beaker, a little quantity of distilled water was added, to this 5 ml of propylene glycol and 1.8 ml of polysorbate 80 were added and mixed well using a magnetic stirrer**.** Obtained powder extracts was added to propylene glycol and polysorbate 80 dispersion, to this preservative (methyl paraben and propyl paraben) and sweetening agent (saccharin sodium) were added. Then a few drops of peppermint oil were added as a flavouring agent. Final volume was made by the remaining quantity of distilled water.

Procedure: All the subjects were briefed about the purpose of the study and informed consent was taken. A medical history & clinical examination was conducted. Randomization was done by using chit method. Relevant information from each case selected was recorded in case history proforma designed for the study. Subjects were recruited into 2 groups: A minimum of 15 subjects was taken per group.

1. Group A – Scaling + Cinnamon *(Cinnamomum zeylanicum)* mouthwash (Test).
2. Group B – Scaling + Chlorhexidine mouthwash (Control).

The control and the test agents are dispensed in a bottle with a code by a non-participant. The subjects were advised to routinely perform mouthwash every day for 2-3 mins in the morning in adjunct to their oral hygiene routine for 30days. Clinical efficacy measurements were

assessed at baseline and 30th day. Microbiological analysis was done at baseline and 30th day.

Following clinical parameters are assessed (at baseline and 30th day)

1. Plaque index-PI (Sillness and Loe, 1964).
2. Gingival index-GI (Loe H and Silness J, 1963).
3. Sulcus bleeding index-SBI (Muhleman H R, 1971).
4. Plaque sample collection for gram staining.

Microbial sampling

At baseline and 30th day, the subgingival plaque samples were obtained from the pockets using sterile Gracey curettes, and transferred and spread onto two clean sterile microscopic slides, and the slides were then stained with Gram stain for microbiological examination in Department of Oral Pathology, Sri Hasanamba Dental College and Hospital. Gram-stained slides were used to make a reliable semiquantitative assessment of morphologically-different types of bacteria. Each slide was examined with a bright field microscope at 100× magnification. Gram-positive and -negative cocci and rods were counted in five randomly-selected microscopic fields.[9] Microbiological status was coded in grades as: (a) less than five colonies organisms, +; (b) five to 15 colonies of organisms, ++; (c) 15–20 colonies of organisms, +++; (d) >20 colonies of organisms, ++++.

Statistical analysis

Statistical analysis was done using SPSS software version 20 and Microsoft excel version 2007. Changes in the clinical and microbiological parameters were assessed over a period of 1 month using descriptive statistics, unpaired ‘t’ test, chi square test and paired sample‘t’ test.

RESULTS

There was no statistical significant difference in mean plaque index score between the groups at baseline (p= 0.978) (Table 1). The PI difference in the mean plaque index score from baseline to 1 month in the Group A is 0.452 and in group B is 0.486 which is statistically significant (p<0.001) (Table 2) (Figure 2).

Table 1: Intergroup comparison of PI, SBI and GI score at baseline and one month.

Unpaired t test					
	GROUP	**N**	**Mean**	**Std. Dev**	**P**
PI BL	GROUP A	15	1.323	0.2326	0.978
	GROUP B	15	1.321	0.1468	
PI 1 MONTH	GROUP A	15	0.870	0.1324	0.495
	GROUP B	15	0.835	0.1470	
SBI BL	GROUP A	15	1.208	0.1619	0.482
	GROUP B	15	1.261	0.2357	
SBI 1 MONTH	GROUP A	15	0.828	0.1650	0.797
	GROUP B	15	0.815	0.1145	
GI BL	GROUP A	15	1.202	0.1850	0.791
	GROUP B	15	1.220	0.1960	
GI 1 MONTH	GROUP A	15	0.813	0.1557	0.384
	GROUP B	15	0.860	0.1323	

Table 2: Inter group difference of PI, SBI & GI scores.

Unpaired t test					
	GROUP	**N**	**Mean**	**Std. Dev**	**P**
PI DIFFERENCE	GROUP A	15	0.452	0.186	0.613
	GROUP B	15	0.486	0.169	
SBI DIFFERENCE	GROUP A	15	0.379	0.107	0.176
	GROUP B	15	0.446	0.149	
GI DIFFERENCE	GROUP A	15	0.388	0.174	0.642
	GROUP B	15	0.360	0.151	

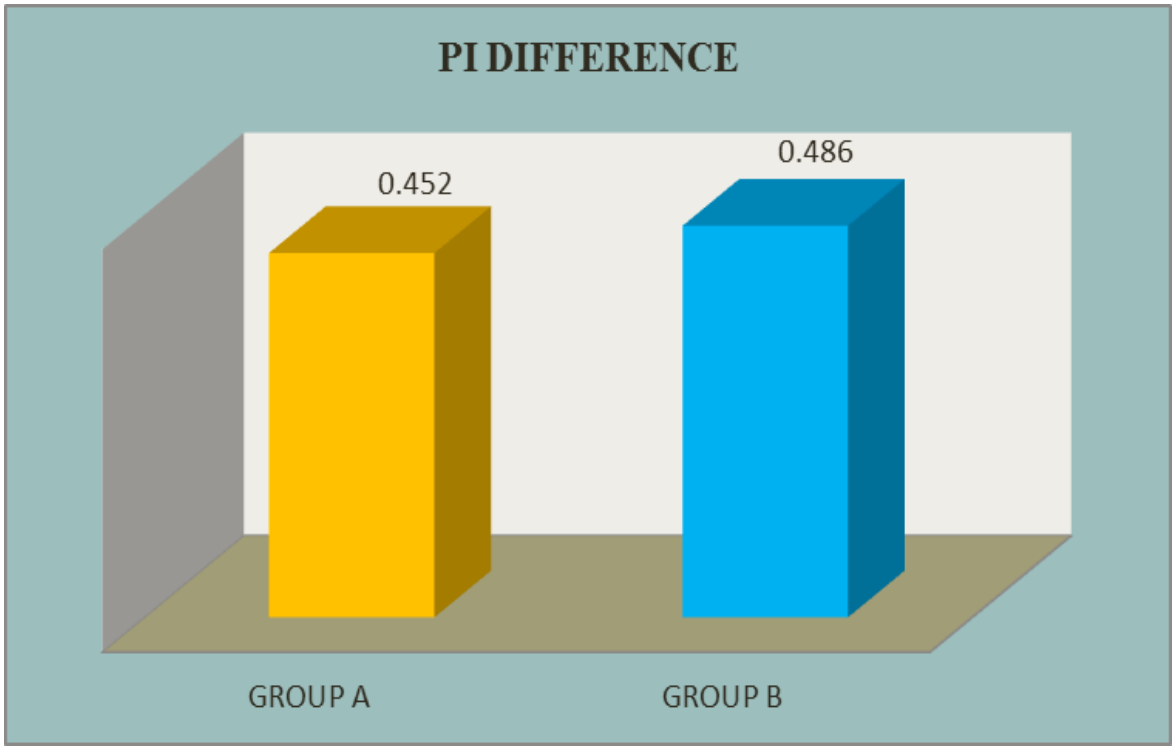

Figure 2: PI difference for both the groups.

No statistical significant difference in mean sulcular bleeding index score between the groups at baseline (p= 0.482) and also after 1 month (p=0.797) (Table 1)**.** The SBI difference in the mean SBI score from baseline to 1 month in the Group A is 0.379 and in the Group B is 0.446 which is statistically significant (p<0.001) (Table 2) (Figure 3).

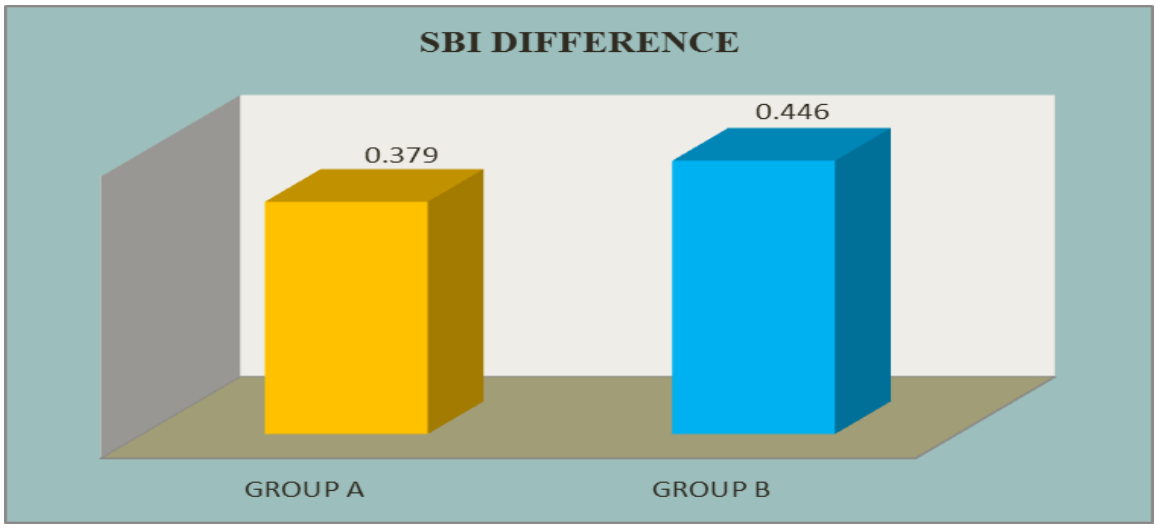

Figure 3: SBI difference for both the groups.

There was no statistical significant difference in mean gingival index score between the groups at baseline (p= 0.791) (Table 1). The GI difference in the mean GI score from baseline to 1 month in the Group A is 0.388 and in the Group B is 0.360 which is statistically significant (p<0.001) (Table 2) (Figure 4).

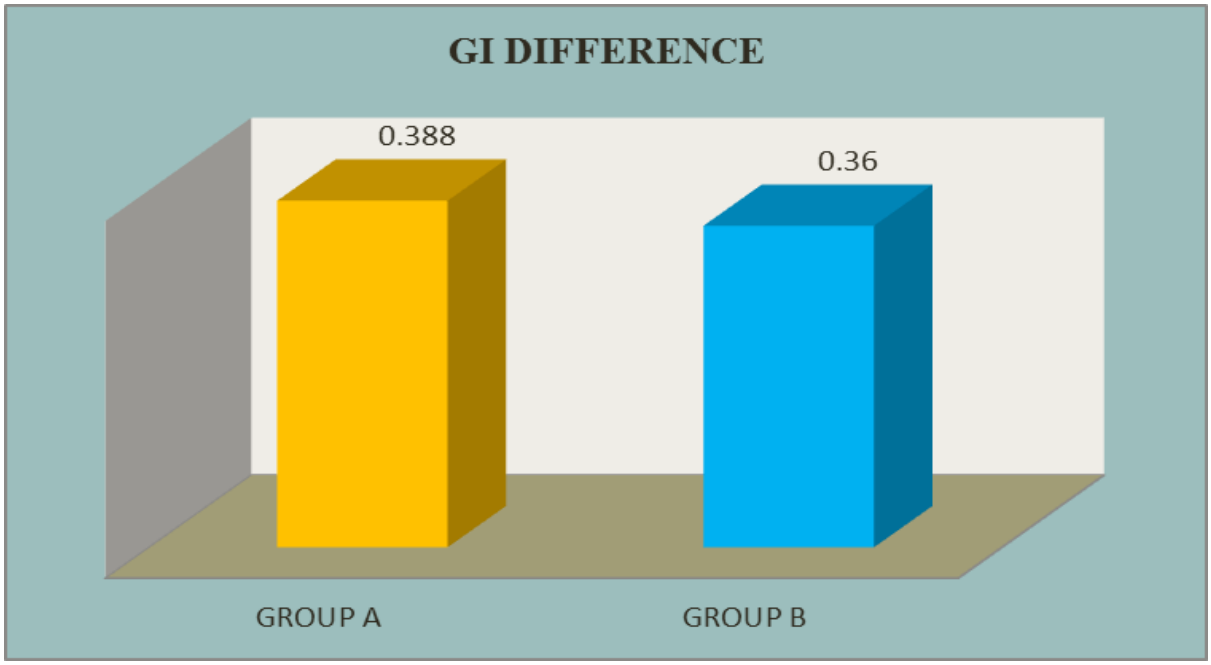

Figure 4: GI difference for both the groups.

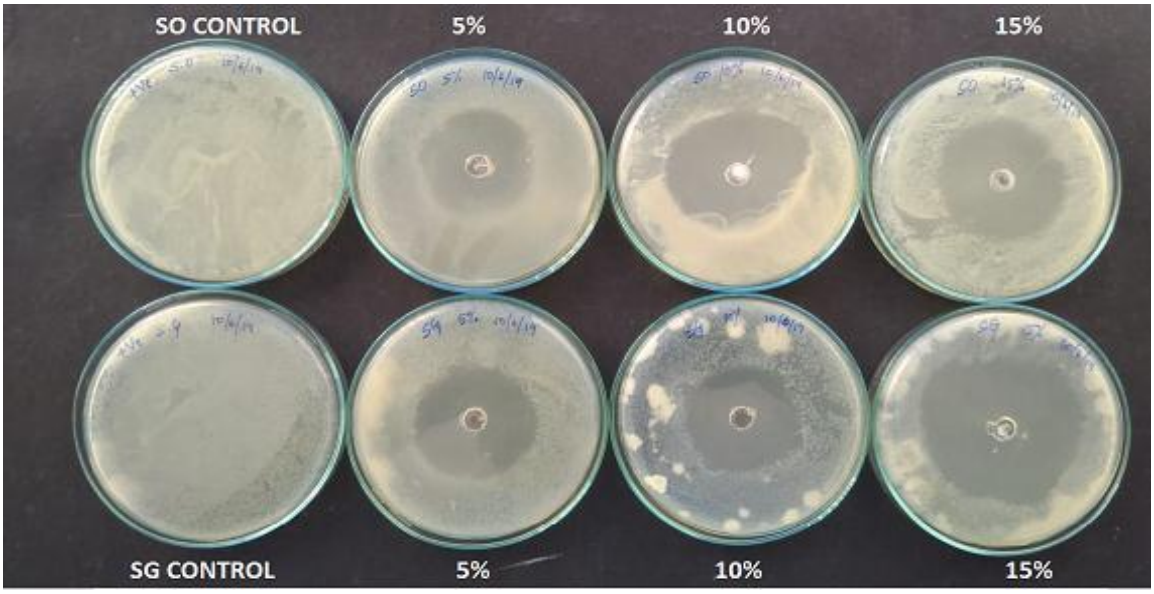

Figure 5: Antimicrobial activity shown by the test samples as zone of inhibition against Streptococcus oralis and Streptococcus gordonii.

Analysis of microbiological parameters

Gram staining (Table 3 and 4)

When intragroup comparisons were done for Gram positive and -negative cocci and bacilli, there was a significant ($P < 0.05$) mean reduction in the number of organisms in both groups A and B. There was no significant difference in intergroup comparison after 30 days (Figure 6 and 7).

Table 3: Showing proportion of subjects with difference in Gram Positive microbial score from baseline to one month in Group A and Group B.

Chi-Square Tests							
GROUP				**GS G + VE 1 MONTH**			
				1+	2+	3+	
GROUP A	GS G + VE BL	2+	Count	2	6	0	0.129
			%	100.0%	50.0%	0.0%	
		3+	Count	0	6	1	
			%	0.0%	50.0%	100.0%	
	Total		Count	2	12	1	
			%	100.0%	100.0%	100.0%	
GROUP B	GS G + VE BL	1+	Count	3	0	0	0.023
			%	37.5%	0.0%	0.0%	
		2+	Count	5	3	0	
			%	62.5%	50.0%	0.0%	
		3+	Count	0	3	1	
			%	0.0%	50.0%	100.0%	
	Total		Count	8	6	1	
			%	100.0%	100.0%	100.0%	

Table 4: Showing proportion of subjects with difference in Gram Negative microbial score from baseline to one month in Group A and Group B.

Chi-Square Tests						
GROUP				GS G - VE 1 MONTH		
				1+	2+	
GROUP A	GS G - VE BL	1+	Count	4	1	0.099
			%	50.0%	14.3%	
		2+	Count	4	4	
			%	50.0%	57.1%	
		3+	Count	0	2	
			%	0.0%	28.6%	
	Total		Count	8	7	
			%	100.0%	100.0%	
GROUP B	GS G - VE BL	1+	Count	7	0	0.005
			%	70.0%	0.0%	
		2+	Count	3	3	
			%	30.0%	60.0%	
		3+	Count	0	2	
			%	0.0%	40.0%	
	Total		Count	10	5	
			%	100.0%	100.0%	

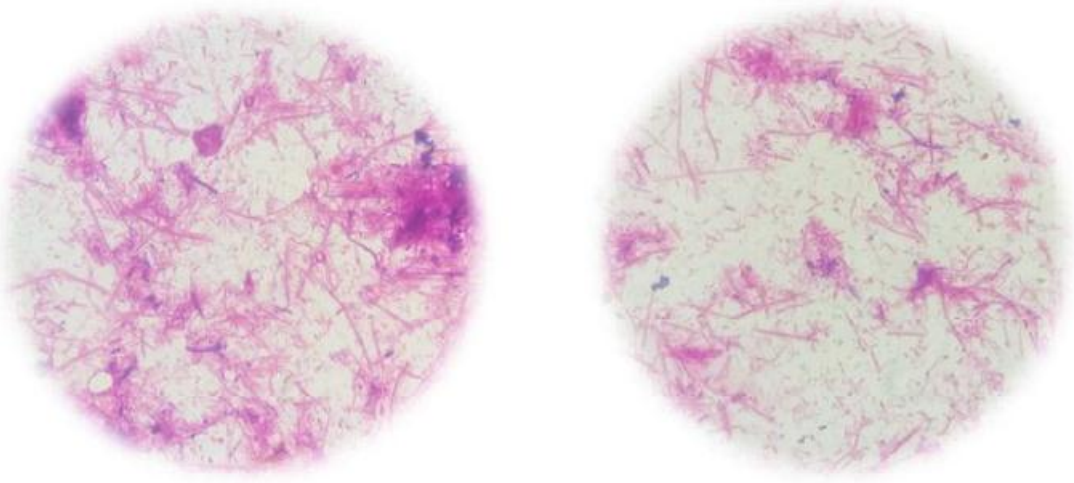

Figure 6: Picture of Microscopic slide of Gram's staining (Group A pre and post-operative).

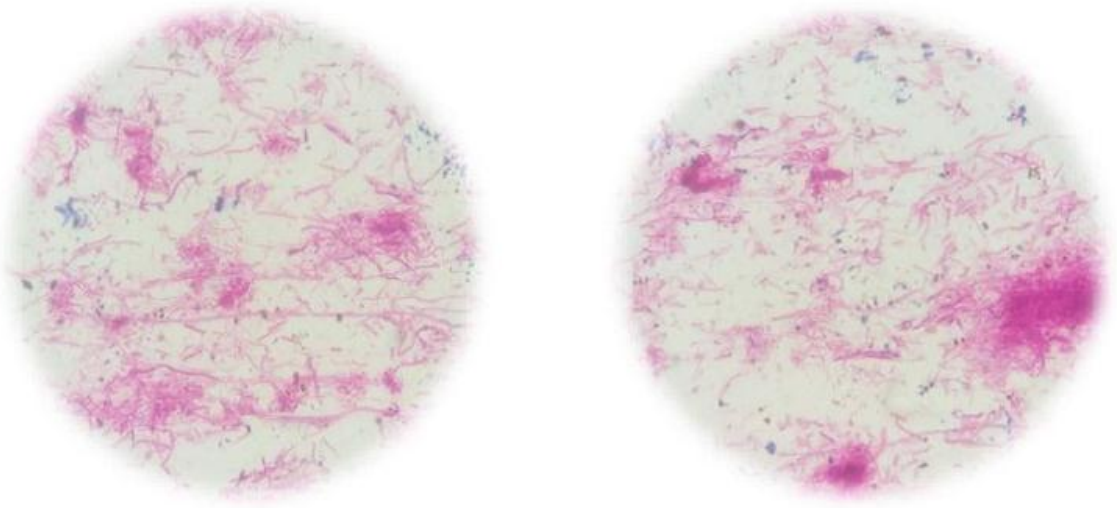

Figure 7: Picture of Microscopic slide of Gram's staining (Group B pre and post-operative).

DISCUSSION

Natural remedies have long history of being used in the treatment of gingival diseases. Periodontal diseases are essentially caused by the microorganisms present in dental plaque thereby reducing the oral microbial load of the plaque biofilm can help in controlling these diseases. Since the advent of antibiotics and the realization that bacteria are the possible causative agents of the major dental diseases, caries and periodontal disease, plaque reduction has been the important aspect of preventive dentistry.[10]

Both the mechanical and chemical adjuncts are used for the prevention and removal of plaque accumulation. Mechanical plaque control must be accompanied by chemical plaque control aids as the majority of the population is not able to perform mechanical plaque removal effectively. Although many antimicrobial agents were tested and found suitable for plaque control, only few possessed clinical efficacy. The reason being, many of these agents lack the property of substantivity and lack efficacy against microorganisms. In this context, chlorhexidine is considered as a gold standard against which the efficacies of other antimicrobial agents are compared. Though chlorhexidine is considered as the gold standard, there are few side effects associated with the drug. The first and the most common side effect being the brownish discoloration of the teeth, restoration and tongue. Staining caused by chlorhexidine is not usually removed by brushing with normal toothpaste.[11] The second being impaired taste sensation.[12] The third being precipitation of calculus formation. There is some evidence that 0.2% chlorhexidine mouth wash has a role in calculus formation.[13] So,

taking into consideration all the side effects of chlorhexidine, herbal mouthwashes are becoming more popular due to the spread in the awareness of the effect of complementary and alternative medicine. It is also due to the belief that alternative medicine is with less side effects.

Cinnamon is one of the main herbs used extensively for treatment of several conditions due to its various medicinal properties such as anti-inflammatory, anti-microbial, anti-oxidant, anti-diabetic, lipid lowering activity etc.[14,15] The in vitro study conducted by Fani and Kohanteb (2011), Gupta et al. (2011), Ayfer and Ozlem Turgay (2003) and Ohara et al. (2007) showed that cinnamon has strong inhibitory activity on Streptococcus mutans, which is the major causative bacteria of dental plaque.[16]

In this study, cinnamon extract was tested against 0.2% chlorhexidine. Cinnamon extract may act as a good oral hygiene product because of its antimicrobial and anti-inflammatory activity. Since, there are fewer studies regarding the efficacy of cinnamon as mouthwash in treatment of gingivitis, this study is carried out to assess the efficacy of 10% cinnamon mouthwash in treatment of gingivitis and to compare it with 0.2% chlorhexidine mouthwash. There was a significant difference in the clinical level of dental plaque, bleeding and gingival index in both cinnamon and chlorhexidine mouthwash groups before and after the experimental period.

A total of 30 subjects were randomly assigned into two groups with 15 subjects in each group. The two groups were, Group A – Scaling + Cinnamon mouthwash and Group B- Scaling + Chlorhexidine mouthwash. The clinical parameters (PI, SBI, GI) were assessed and microbiological analysis (Gram staining) was done at baseline and after one month. The results of these two groups on plaque level and gingivitis was compared with the only study done by Gupta et al in which they compared the effect of cinnamon extract, chlorhexidine mouthwash and placebo on dental plaque level and gingivitis as no other studies have been reported in literature. The results on sulcular bleeding index and microbiologic parameters could not be compared with any other studies, as no studies have been reported in literature that have tried to assess the same effect.

In the present study, the intragroup mean score of PI done using paired ‘t’ test in both the groups i.e Group A and B was statistically significant when compared from baseline to 1 month. Intergroup comparison had shown that mean plaque reduction of Group B is higher

than that of Group A from baseline to one month but it was not statistically significant (p=0.613). Though chlorhexidine mouthwash was found to be more effective at the end of 1 month, scaling along with cinnamon mouthwash is also significantly effective in reducing plaque score from baseline to one month. This was in accordance with the study conducted by Gupta D et al 2015, which showed that cinnamon mouthwash was effective in reducing the plaque scores at the end of one month when combined with scaling.[17] Statistically significant reduction in SBI was observed in both the groups from baseline to one month (p<0.001). When comparing intergroup differences of SBI, Group B had higher reduction when compared to Group A but it was not statistically significant (p=0.176). Statistically significant reduction in GI was observed in both the groups from baseline to one month (p<0.001). Intergroup comparison of GI differences showed that group A had higher reduction compared to group B but it was not statistically significant (p=0.642). Hence cinnamon mouthwash had better reduction of gingival index than chlorhexidine which is not in accordance with the study done by Gupta et al., where reduction of GI was more in chlorhexidine group. Gram's staining showed a quantitative reduction in the gram positive and gram negative organisms. There was a reduction in the scoring level from baseline to one month for both the groups but it was not statistically significant.

CONCLUSION

10% cinnamon mouthwash is almost equally effective as 0.2% chlorhexidine in treating chronic generalised gingivitis. There was significant decrease in all the clinical parameters of gingivitis compared to the baseline.

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