

COMPARATIVE EVALUATION OF EFFICACY OF ACACIA CATECHU WILLD AND CHLORHEXIDINE AS MOUTHWASH ON GINGIVITIS – A CLINICOMICROBIOLOGICAL STUDY

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ABSTRACT

Background: A mouthwash is a medicated liquid which is held in the mouth and swished by the action of perioral musculature to eliminate the oral pathogens. *A. catechu* extract may act as a good oral hygiene product because of its antimicrobial and anti-inflammatory activity. Aim of the study was to evaluate the effects of *A. catechu* bark extract as mouthwash, compared to 0.2% CHX, on plaque and sulcular bleeding levels in 18-45 year old gingivitis patients. **Materials and methods:** An in vitro analysis was done to determine the MIC of *A. catechu* on *S. mutans* and *S. aureus* which was found to be 200mg/ml (20%) concentration which was further used for the *in vivo* study. In this randomized controlled clinical trial, 40 subjects were randomly assigned to two groups with 20 subjects in each group, Group A-

Scaling + 20% *A. catechu* mouthwash and Group B- Scaling + 0.2% Chlorhexidine mouthwash. Clinical parameters like Plaque index (PI), Gingival index (GI), Sulcus Bleeding Index (SBI) and Microbiological analysis for the counts of *S. mutans* and *S. aureus* colonies were evaluated at baseline and 15th day. **Results:** Intergroup comparison showed statistically non-significant difference between the groups in reduction of scoring level from baseline to 15th day. The microbiological analysis also showed a statistically significant reduction in

colonies of the *S. mutans* and *S. aureus* in both the groups at 15th day ($p < 0.001$). **Conclusion:** 20% Acacia catechu mouthwash is almost equally effective as 0.2% chlorhexidine in treating chronic generalised gingivitis.

KEYWORDS: Acacia catechu, Mouthwash, Gingivitis, MIC, PI, GI, SBI.

INTRODUCTION

Dental caries and Periodontal diseases, the two arch criminals of oral cavity, are essentially caused by the micro-organisms present in dental plaque.^[1] Dental plaque plays an important role in the development of dental caries and periodontal disease, which results in both dysfunction and loss of tooth.^[2] Periodontal diseases (gingivitis and periodontitis) are considered inflammatory diseases of microbiological origin. Although not all patients with gingivitis will develop periodontitis, the management of gingivitis is considered both a primary prevention strategy for periodontitis and secondary for recurrent periodontitis.^[3]

Gingivitis is the inflammation of gingiva without apical migration of junctional epithelium which, unless treated, will lead to periodontitis in susceptible patients.^[4] Mechanical removal of plaque via tooth brush and use of dental floss has been considered as an effective method in controlling gingivitis.^[5] Nevertheless, adequate time of brushing, efficient cleaning of all tooth surfaces and regular oral hygiene is hard to achieve in every individual due to variations in oral health practices which accounts for high prevalence of gingivitis.^[6] Therefore, additional approaches such as dentifrices and mouthwashes containing chemical or herbal agents are suggested.^[7]

A mouthwash is a medicated liquid which is held in the mouth and swished by the action of perioral musculature to eliminate the oral pathogens.^[8] Mouthwashes are to be used only as an adjunct to mechanical plaque control measures. These chemical methods of reducing plaque are appealing as they can provide significant benefits to patients who cannot maintain adequate mechanical plaque control.^[9] Among the plethora of oral hygiene products available, Chlorhexidine has been the mouthwash of choice owing to its dramatic therapeutic effect.^[10,11]

CHX is the most effective and most thoroughly tested antiplaque and antigingivitis agent known today.^[12] Though very effective, it also has certain side effects like brown discolouration of the teeth, oral mucosal erosion and bitter taste. Hence, there is a need of an

alternative medicine that could provide a product enmeshed within the traditional Indian set up and is also safe and economical.^[13]

Acacia catechu Willd (Khadir) belonging to Family-Fabaceae and subfamily-Mimosoideae is a historical plant which has a valuable importance because of its medicinal properties. *A. catechu* exhibits various pharmacological effects like anti-inflammatory, anti-diarrhoeal, hypoglycemic, antioxidant, hepatoprotective, antipyretic, antimicrobial, anticancer, antibacterial, anti-ulcer and antisecretory activities, sore throat and wound healing etc. *A. catechu* is also useful as a topical agent for sore gums and mouth ulcers. Thus the plant has diverse pharmacological actions.^[14]

A. catechu extract may act as a good oral hygiene product because of its antimicrobial and anti-inflammatory activity. Literature review revealed very few *in vivo* studies worldwide assessing the effects of *A. catechu* extracts as mouthwash in treatment of Gingivitis. Hence, present study was undertaken to ferret out the effects of *A. catechu* bark extract as mouthwash, compared to 0.2% CHX, on plaque and sulcular bleeding levels in 18-45 year old gingivitis patients.

MATERIALS AND METHODS

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 coin toss method. The study design was approved by the Institutional Ethics Committee of Sri Hasanamba Dental College and Hospital, Hassan, Karnataka.

Inclusion Criteria

Systemically healthy subjects, between 18-45 years of age, with minimum of 10 gradable teeth, who were willing to comply with the study protocol and had clinical signs of gingivitis were included in the study.

Exclusion Criteria

Subjects with any systemic diseases/conditions and on any medication which can affect periodontium were excluded. Smokers, pregnant and lactating mothers were excluded from

the study. Patients who have received any periodontal therapy in the last 6 months were also excluded from the study.

In-vitro Analysis

The antimicrobial effect of *A. catechu* on primary colonisers such as *Streptococcus mutans* and *Staphylococcus aureus* was analysed, by determining the Minimum Inhibitory Concentration of the drug. The sample solutions of 5%, 10%, 15%, 20% and 25% were prepared for testing the MIC of the drug based on an in vitro study.

Bacterial sample preparation

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

Antibacterial activity assay

Antibacterial susceptibility test of the plant extract was done against *S. aureus* and *S. mutans*. Luria bertani agar medium for *S. aureus* and Brain heart infusion agar medium for *S. mutans* were prepared. Wells were punctured into the agar medium with a sterilized cork borer, Spread plate technique was carried out with pre-cultured *S. aureus* and *S. mutans*. Samples of 5%, 10%, 15%, 20% and 25% concentrations of 100µl were loaded into the wells. Then the plates were incubated at 37°C for 24- 48hrs after the incubation, the zones of inhibition were measured and tabulated. From the analysis it was concluded that the sample showed antimicrobial activity against both *S. aureus* and *S. mutans*. Above 15%, the plant sample showed good antibacterial effect against both the microorganisms (Figure 2). Hence, 20% *A. catechu* was used for preparing the final mouthwash.

Preparation of *A. catechu* mouthwash

A. catechu bark extract powder was obtained from SDM college of Ayurveda and Hospital, Hassan. The pharmacological preparation was also done by the same institution.

Procedure for mouthwash preparation

In this method, herbal mouthwash (20% w/w) was prepared using *A. catechu* powder. Firstly, 20g of *Acacia catechu* powder was weighed and transferred into 200 ml sterile beaker. Then, 20 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 10 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. Medical history was taken and clinical examination was conducted. Randomization was done by using coin toss 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 20 subjects were taken per group.

1. Group A – Scaling + *A. catechu* mouthwash (TEST)
2. Group B – Scaling + CHX mouthwash (CONTROL)

The subjects were advised to routinely use mouthwash every day for 2-3 minutes in the morning as an adjunct to their oral hygiene routine for 14 days. Clinical efficacy measurements were assessed at baseline and on 15th day. Microbiological samples were obtained at baseline and on 15th day.

Clinical parameters assessed (at baseline and 15th 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) Saliva sample collection for microbiological analysis.

Microbiological analysis

Saliva sample was collected in a sterile container. The participants were instructed to spit into the container and saliva was stored in a refrigerator at 2-8°C. 1 mL of sample was placed onto 5% sheep blood agar. With a sterile swab, lawn culture was performed. Agar medium was incubated in a candle jar to provide anaerobic condition for the growth of *S. mutans*. The plates were observed for the bacterial growth. Colonies were counted using Digital colony counter. Gram staining was performed on different colony types. After Gram staining, Gram +ve cocci were further subjected to preliminary test like Catalase test. The catalase +ve cocci were further subjected to coagulase test. Those which were coagulase +ve were confirmed to

be *S. aureus*. Those which were catalase –ve were subjected to confirmatory tests for *S. mutans*.

STATISTICAL ANALYSIS

Statistical analysis was done using Microsoft excel version 2010. 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

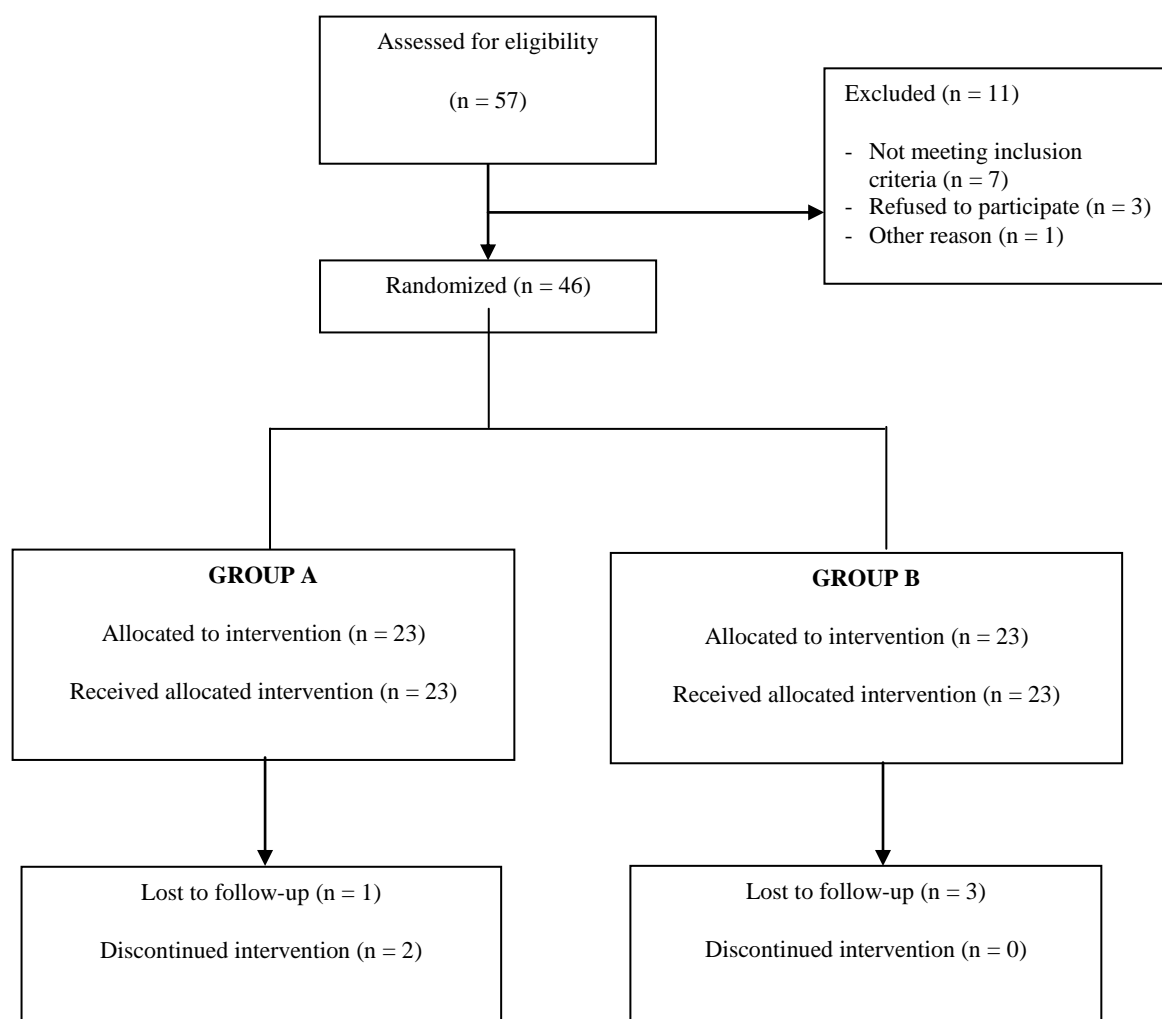
Table 1 and Graphs 1, 2 and 3 shows the intergroup comparison of PI, SBI and GI score at baseline and 15th day. There was no statistically significant difference in mean plaque index score between the groups at baseline ($p=0.969$) or at 15th day ($p=0.060$). The mean sulcular bleeding index score at baseline ($p=0.787$) and at 15th day ($p=0.654$) also showed no statistically significant difference between the groups. No statistically significant difference in mean gingival index score was found between the groups at baseline ($p=0.267$) or at 15th day ($p=0.022$).

Table 2 shows intragroup comparison of PI, SBI and GI scores using paired t test of Group A and Group B. The mean plaque index score at baseline in Group A was 1.2855 ± 0.1895 , and on 15th day was 0.7455 ± 0.13496 which is statistically significant ($p<0.001$). The mean plaque index score at baseline in Group B was 1.2835 ± 0.12304 , and at 15th day was 0.8235 ± 0.11851 which is statistically significant ($p<0.001$). The mean sulcular bleeding index score at baseline in Group A was 1.1935 ± 0.14576 , and on 15th day was 0.658 ± 0.24352 which is statistically significant ($p<0.001$). The mean sulcular bleeding index score at baseline in Group B was 1.2070 ± 0.16743 , and on 15th day was 0.688 ± 0.16929 which is statistically significant ($p<0.001$). The mean Gingival index score at baseline in Group A was 1.156 ± 0.10246 , and on 15th day was 0.6575 ± 0.20527 which is statistically significant ($p<0.001$). The mean sulcular bleeding index score at baseline in Group B was 1.2085 ± 0.18167 , and on 15th day was 0.8070 ± 0.1909 which is statistically significant ($p<0.001$).

Table 3 and Graphs 4, 5 shows Proportion of subjects with difference in *S. aureus* colony counts from baseline to 15th day in Group A and B. In Group A, 1 subject having level 1+ at baseline remained at level 1+ on 15th day. Out of 10 subjects having level 2+ at baseline 5 subjects reduced to 1+ and 5 subjects remained at 2+ on 15th day. Among 9 subjects of level 3+ at baseline, 3 subjects have reduced to level 1+ and 6 subjects reduced to level 2+ on 15th

day. In Group B, out of 4 samples of level 1+ at baseline, all remained at level 1+ on 15th day. Out of 10 subjects of level 2+ at baseline, 8 subjects reduced to level 1+ and 2 subjects remained at level 2+ on 15th day. Of the 6 subjects of level 3+ at baseline 2 reduced to level 1+ and 4 reduced to 2+ level on 15th day.

Table 4 and Graphs 6,7 shows Proportion of subjects with difference in *S. mutans* colony counts from baseline to 15th day in Group A and B. In Group A, out of 4 subjects having level 1+ at baseline 2 remained at level 1+ and 2 showed level 2+ on 15th day. Out of 11 subjects having level 2+ at baseline 7 subjects reduced to 1+ and 4 subjects remained at 2+ on 15th day. Among 5 subjects of level 3+ at baseline, 2 subjects have reduced to level 1+ and 3 subjects reduced to level 2+ on 15th day. In Group B, out of 3 samples of level 1+ at baseline, all remained at level 1+ on 15th day. Out of 9 subjects of level 2+ at baseline, 8 subjects reduced to level 1+ and 1 subject remained at level 2+ on 15th day. Of the 8 subjects of level 3+ at baseline 3 reduced to level 1+ and 5 reduced to 2+ level on 15th day.



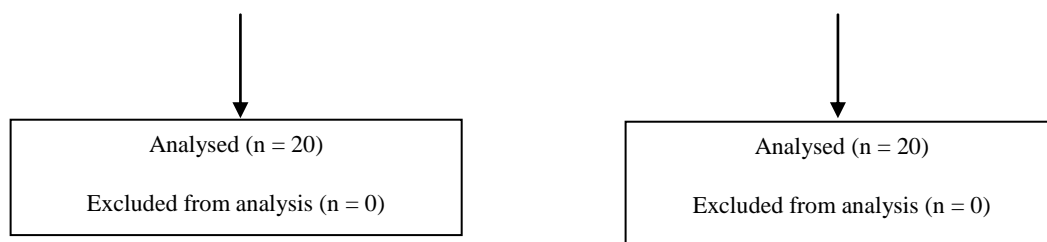
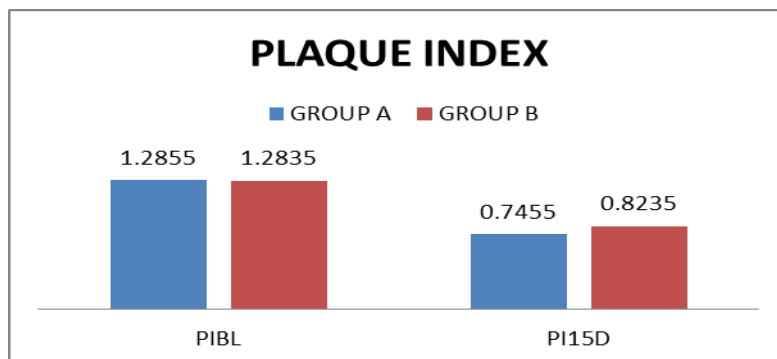
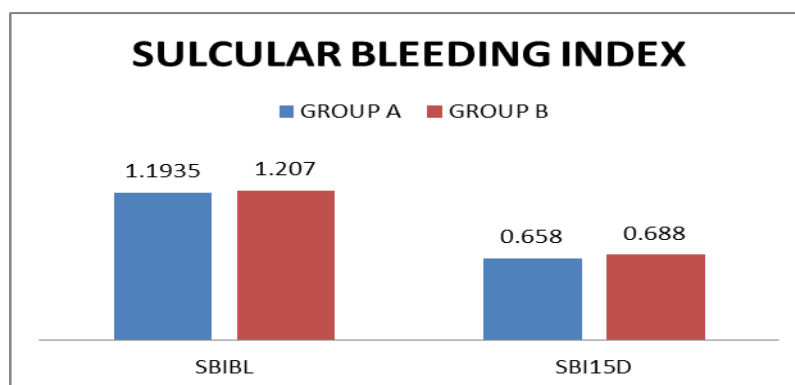
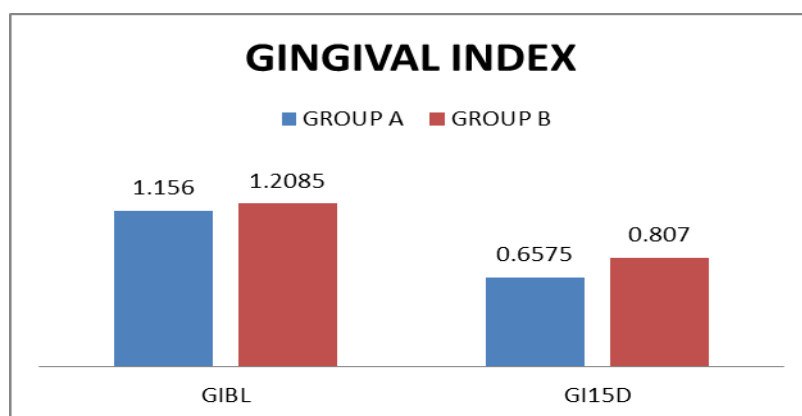


Figure 1: Consort chart.

Table 1: Intergroup comparison of PI, SBI and GI score at baseline and 15th day.

Group Statistics					
	GROUP	N	Mean	Std. Deviation	p
PIBL	GROUP A	20	1.2855	0.18950	0.969
	GROUP B	20	1.2835	0.12304	
PI15D	GROUP A	20	0.7455	0.13496	0.060
	GROUP B	20	0.8235	0.11851	
SBIBL	GROUP A	20	1.1935	0.14576	0.787
	GROUP B	20	1.2070	0.16743	
SBI15D	GROUP A	20	0.6580	0.24352	0.654
	GROUP B	20	0.6880	0.16929	
GIBL	GROUP A	20	1.1560	0.10246	0.267
	GROUP B	20	1.2085	0.18167	
GI15D	GROUP A	20	0.6575	0.20527	0.022
	GROUP B	20	0.8070	0.19090	

Graph 1: PI index at baseline and at 15th day for both the groups.Graph 2: SBI at baseline and at 15th day for both the groups.



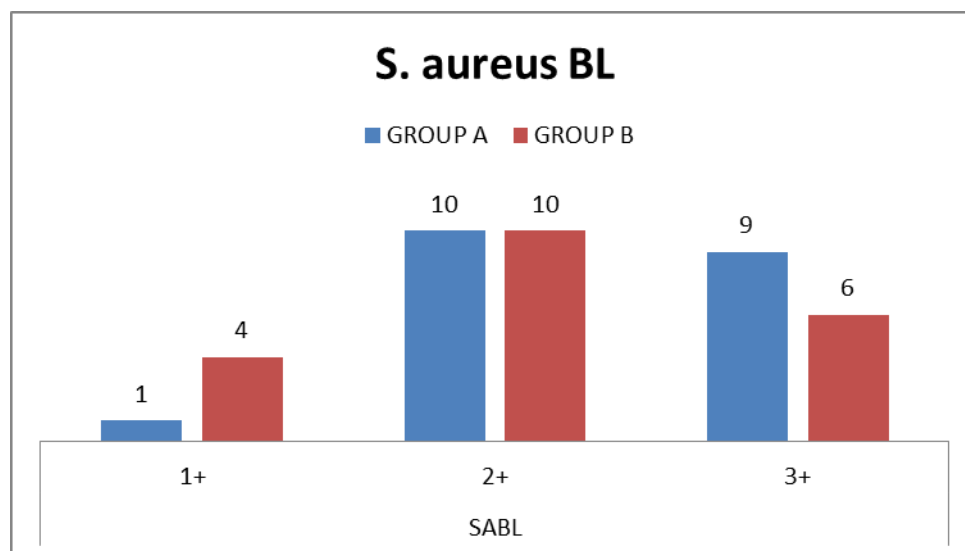
Graph 3: GI index at baseline and at 15th day for both the groups.

Table 2: Intragroup comparison of PI, SBI and GI scores using paired t test of Group A & Group B.

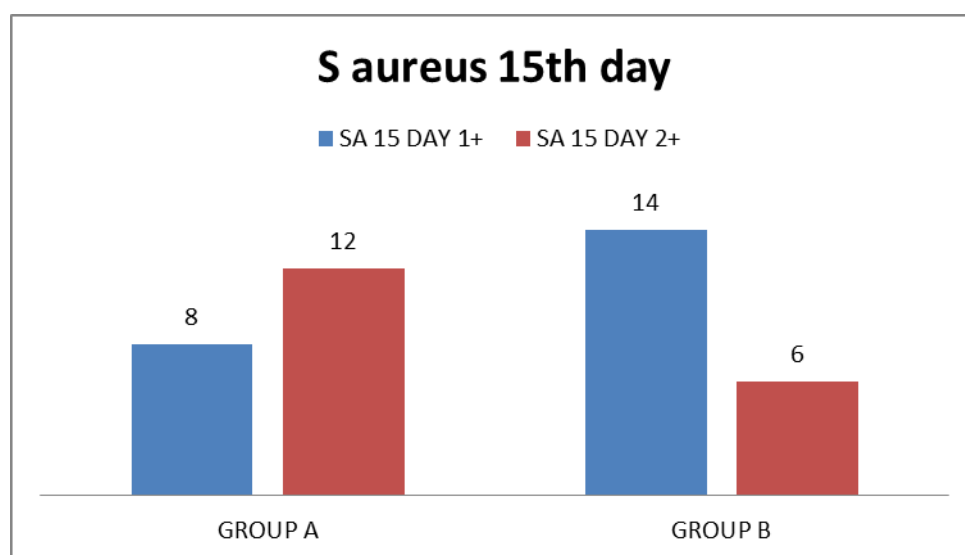
GROUPS			Mean	N	Std Deviation	p
GROUP A	Pair 1	PIBL	1.2855	20	0.18950	<0.001
		PI15D	0.7455	20	0.13496	
	Pair 2	SBIBL	1.1935	20	0.14576	<0.001
		SBI15D	0.6580	20	0.24352	
	Pair 3	GIBL	1.1560	20	0.10246	<0.001
		GI15D	0.6575	20	0.20527	
GROUP B	Pair 1	PIBL	1.2835	20	0.12304	<0.001
		PI15D	0.8235	20	0.11851	
	Pair 2	SBIBL	1.2070	20	0.16743	<0.001
		SBI15D	0.6880	20	0.16929	
	Pair 3	GIBL	1.2085	20	0.18167	<0.001
		GI15D	0.8070	20	0.19090	

Table 3: Proportion of subjects with difference in *S. aureus* colony counts from baseline to 15th day in Group A and Group B.

GROUP				SA15DAY		p
				1+	2+	
GROUP A	SABL	1+	Count	0	1	0.535
			% within SA15DAY	0.0%	8.3%	
		2+	Count	5	5	
			% within SA15DAY	62.5%	41.7%	
		3+	Count	3	6	
			% within SA15DAY	37.5%	50.0%	
GROUP B	SABL	1+	Count	4	0	0.049
			% within SA15DAY	28.6%	0.0%	
		2+	Count	8	2	
			% within SA15DAY	57.1%	33.3%	
		3+	Count	2	4	
			% within SA15DAY	14.3%	66.7%	



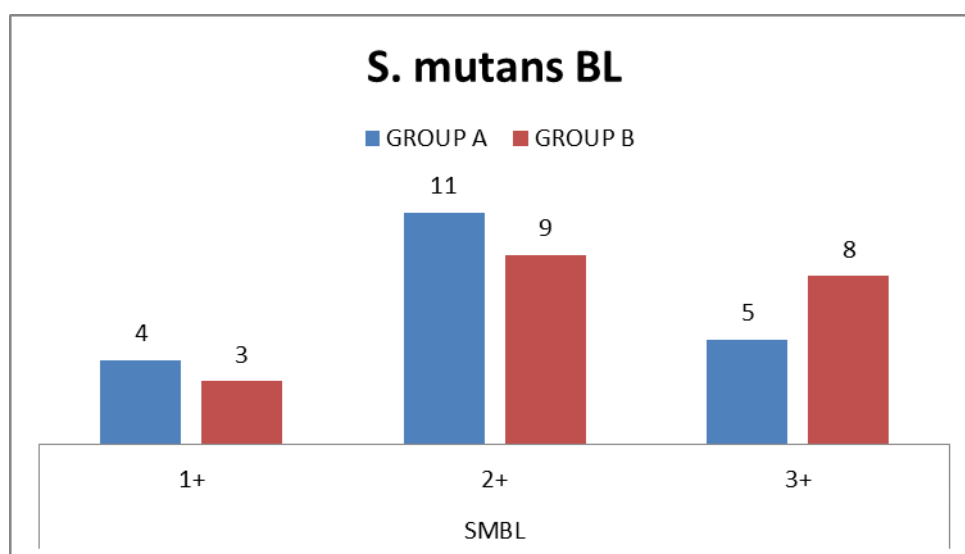
Graph 4: Difference of proportions of the study groups with respect to *S. aureus* at baseline.



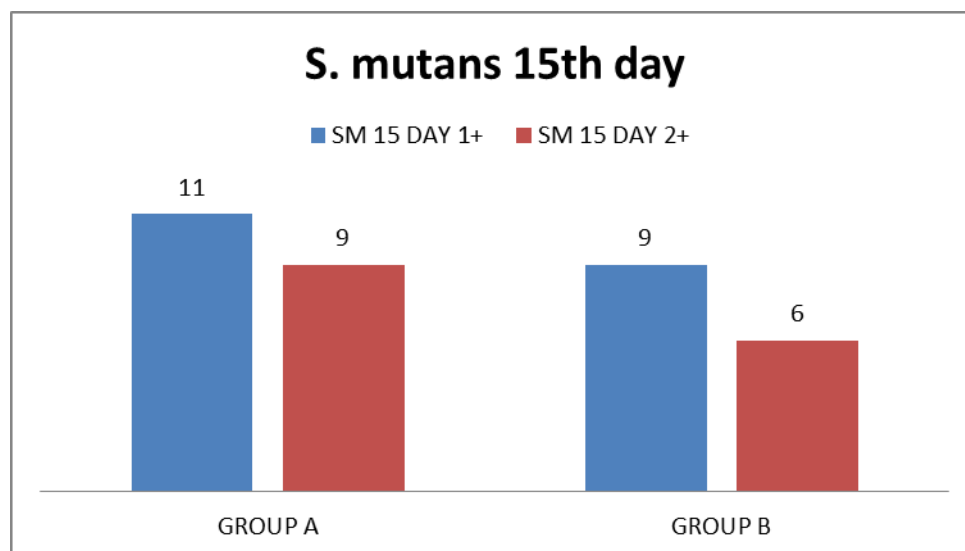
Graph 5: Difference of proportions of the study groups with respect to *S. aureus* at 15th day.

Table 4: Proportion of subjects with difference in *S. mutans* colony counts from baseline to 15th day in Group A and Group B.

GROUP				SM15DAY		p
				1+	2+	
GROUP A	SMBL	1+	Count	2	2	0.662
			% within SM15DAY	18.2%	22.2%	
		2+	Count	7	4	
			% within SM15DAY	63.6%	44.4%	
		3+	Count	2	3	
			% within SM15DAY	18.2%	33.3%	
GROUP B	SMBL	1+	Count	3	0	0.033
			% within SM15DAY	21.4%	0.0%	
		2+	Count	8	1	
			% within SM15DAY	57.1%	16.7%	
		3+	Count	3	5	
			% within SM15DAY	21.4%	83.3%	



Graph 6: Difference of proportions of the study groups with respect to *S. mutans* at baseline.



Graph 7: Difference of proportions of the study groups with respect to *S. mutans* at 15th day.

DISCUSSION

Gingivitis is generally regarded as a site-specific inflammatory condition initiated by dental biofilm accumulation^[2-4] and characterized by gingival redness and edema^[5] and the absence of periodontal attachment loss.^[6] Gingivitis is commonly painless, rarely leads to spontaneous bleeding, and is often characterized by subtle clinical changes, resulting in most patients being unaware of the disease or unable to recognize it.^[7]

Although not all patients with gingivitis will develop periodontitis, the management of gingivitis is considered both a primary prevention strategy for periodontitis and secondary for recurrent periodontitis.^[15] One of the limitations of mechanical plaque control procedures is that they concentrate solely on the hard surfaces of the oral cavity. Although the non-shedding surfaces of the teeth provide an excellent surface for the establishment and growth of biofilms, they represent a relatively small percentage of the total area of the oral cavity (21-23%).^[16] Recent studies have demonstrated that microorganisms involved in the etiology of gingivitis and periodontitis accumulate on several soft tissue surfaces of the mouth, which serve as a source of bacteria for the colonization of tooth surfaces.^[17] Chemical anti-plaque agents present in mouthrinses or dentifrices could reach these soft tissue surfaces, improving the control of biofilm growth on these surfaces and delaying microbial accumulation on teeth. This mechanism was illustrated in a study that examined the rate of biofilm accumulation on teeth after a 3-week preparatory phase that included oral hygiene instructions and frequent professional cleanings.^[18] Chlorhexidine which is considered as gold standard in chemical de-

bridement has its own drawbacks which include staining of teeth, taste alteration and form lesions that are desquamatory and erythematous in nature. After prolonged use, brownish pigmentation of the tooth surface, prosthesis and tongue can be appreciated.^[19] There is also some evidence that 0.2% chlorhexidine mouthwash has a role in calculus formation.^[20] These undesirable side effects limit the long-term use and the patient acceptability of CHX mouthwashes. Thus, the search for alternatives continues, and the focus shifted toward biogenic agents.^[21]

In this study, *A. catechu* extract was tested against 0.2% chlorhexidine. *A. catechu* 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 was carried out to assess the efficacy of 20% 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 *A. catechu* and chlorhexidine mouthwash groups before and after the experimental period.

A total of 40 subjects were randomly assigned into two groups with 20 subjects in each group. The two groups were, Group A – Scaling + *Acacia catechu* mouthwash and Group B- Scaling + Chlorhexidine mouthwash. Clinical parameters (PI, SBI, GI) were assessed and microbiological analysis (Digital colony counting) was done at baseline and after one month. The antiplaque and antigingivitis effects of the two mouthwashes used in our study was similar to a study done by Agarwal DR et al^[22] in which they compared the effect of *A. catechu* extract and chlorhexidine mouthwash on dental plaque level and gingivitis. Another study done by Joshi SG²³ et al assessing the plaque index, sulcular bleeding index and gingival index, as compared to 0.2% Chlorhexidine reported that *Acacia catechu* showed better results than Chlorhexidine. The results on microbiologic parameters could not be compared with any other studies, as no studies have been reported in literature that has 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 14th day. Intergroup comparison had shown that mean plaque reduction of Group B is higher than that of Group A on the 15th day but it was not statistically significant ($p=0.060$). Though chlorhexidine mouthwash was found to be more effective at the end of 14th day, scaling along

with *Acacia catechu* mouthwash was also found to be significantly effective in reducing plaque score from baseline to 14th day. 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 on the 15th day when compared to Group A, but it was not statistically significant ($p = 0.654$). 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 B had higher reduction compared to group A on the 15th day but it was not statistically significant ($p = 0.022$). All the findings were in accordance with the study conducted by Agarwal DR *et al*, which showed that *A. catechu* mouthwash was statistically equally effective in reducing the plaque, sulcular bleeding and gingival scores at the end of two weeks when combined with scaling.²² Digital colony counting showed a significant reduction in the *S. mutans* and *S. aureus* colonies by two weeks in both the groups.

CONCLUSION

20% *Acacia catechu* mouthwash is almost equally effective as 0.2% chlorhexidine in the treatment of chronic generalised gingivitis. There was significant decrease in all the clinical parameters of gingivitis and microbiological analysis compared to the baseline.

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