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Antibacterial efficacy of Neem and Cinnamon mouth rinse on salivary mutans streptococci and its effect on plaque and gingival health among 15–17-year-old students in Madurai city- A Double blind, Randomized Controlled Trial (RCT)

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Abstract

Introduction: Oral deposits such as plaque and calculus have a huge impact on oral health related quality of life. In India oral diseases prevalence is very high, among which dental caries and gingival diseases are non-life threatening and ubiquitous pathologies. mutans strep to cocci being the pioneer culprit in the initiation of oral diseases, any intervention that inhibit their growth and survival will have positive impact on oral health. Antibacterial agents of botanical extracts of neem and cinnamon have been used as mouth rinse are used as alternatives.

Aim and objectives: To compare the Antibacterial efficacy of 5% Neem, 10% Cinnamon and 0.2% Chlor Hexi dine on salivary mutans strep to cocci and its effect on plaque and gingival health.

Materials and methods: It is a randomized, parallel arm, controlled trial designed to compare the effect of 5% Neem 10% Cinnamon mouth rinse with 0.2% Chlor Hexi dine on the salivary mutans strep to cocci and its effect on plaque and gingival health.

Results: There was a statistically significant reduction on salivary mutans streptococci colonies and gingivitis scores in the saliva after using 5% neem and 10% cinnamon mouth rinse ($p<0.05$) Significant difference

was observed between the mutans strep to cocci Count (CFU/ ml) before and after rinsing with 5% neem 10% cinnamon, 0.2% Chlorhexidine.

Conclusion

5% Neem and 10% Cinnamon was considerably efficacious in reducing the mutans streptococci count, plaque and gingivitis significantly, although not superior to the gold standard 0.2% chlor Hexi dine.

Keywords: Neem, Cinnamon, mutans strep to cocci, Gingival health

Introduction

Dental plaque is a general term used for the diverse microbial community found on the surfaces of the tooth. It is embedded in a matrix of polymers which is of salivary and microbial origin. Oral deposits such as plaque and calculus have a huge impact on oral health related quality of life. In India oral diseases prevalence is very high, among which dental caries and gingival diseases are non-life threatening and ubiquitous patho logies[1]. Dental caries is an irreversible disease affecting both organic and inorganic tooth structure and the most significant etiological agent causing dental caries is mutans streptococci. This strain of bacteria is considered as harmful to humans due to the fact that they congregate in large numbers and forms plaques on the tooth surfaces. The presence of mutans streptococci in dental plaque has been reported to be positively associated with dental caries. It is the most predominant of all bacteria growing on the teeth, tongue, labial mucosa, buccal mucosa, and in the saliva of humans. It is mostly found residing in the pits of human teeth and along the gingival crevices. It is the most potent destructive microbe which rapidly ferments sugars to lactic and other organic acids which in turn reduces the salivary pH and initializes demineralization of enamel[2]. Mouth washes are used in dentistry for prevention and curative purpose it disrupts plaque by chemo mechanical action, among which Chlorhexi dine is the most potent Che mother a peutic agent it is considered as gold standard in reducing mutans strep to cocci and in controlling plaque. In lower concentration it is bacterio static and at higher concentration it is bactericidal.

Incidence of its adverse effects such as undesirable taste, teeth discoloration, burning sensation, dryness in the mouth discourages patients to use this mouth wash in longer run, therefore natural alternatives derived from botanicals are being explored.[3] so this study was taken up to formulate with novel, safe and cost-effective mouth rinse for prevention as well as to reduce the prevalence of oral diseases. Available literature shows that there are very few studies comparing the efficacy of neem and cinnamon as an antimicrobial agent, hence an attempt has been made in this less explored path.

AIM

To compare the antibacterial efficacy of 5% Neem and 10% Cinnamon mouth rinse on salivary mutans strep to cocci and to assess its effects on plaque and gingival status.

Objectives

- To assess the efficacy on mutans strep to cocci using 0.12% Chlorhexidine,
- 5% Neem and 10% Cinnamon mouth rinse.
- To assess Plaque and Gingival status in the subjects using 0.12% Chlorhexidine, 5% Neem and 10% Cinnamon mouth rinse.
- To compare the antibacterial efficacy between 0.12% Chlorhexidine, 5% Neem and 10% Cinnamon mouth rinse.
- To compare the antiplaque and anti-gingival effect between 0.12% Chlorhexidine, 5% Neem and 10% Cinnamon mouth rinse.

Materials and methods

This research is a randomized, parallel arm, controlled trial designed to compare the effect of 5% Neem and 10% Cinnamon with 0.2% Chlorhexidine mouth rinse on the mutans sreptococci count in the saliva, gingival health and plaque status. Students aged 15- to 17-year-old (n=102) those who are willing to Participate in the study was Included subjects with at least one active caries lesion, with gingival and or/periodontal disease subjects who brush only once daily was included in the study. Patients who received antibiotic therapy within 3 weeks, subjects with Systemic illness and limited manual dexterity were excluded. A single examiner, the investigator, carried out the clinical examination of all the study subjects involved in the study under artificial light using standardized instruments ADA specification type III examination was followed. Decayed, Missing and Filled teeth index and Decayed, Missing, and Filled surfaces Index, Plaque Index (PI) determine the extent of plaque. Gingival Index (GI) describe the clinical severity of gingival inflammation as well as its location. The Stimulated whole saliva was collected by "draining/ spitting" method. Mutans strep to cocci count of all the samples were estimated. The saliva containers were quickly closed in order to avoid contamination and stored in cold storage box and were transported to the Microbiological lab within 1-2 hours. M259 - The agar plates streaked with the collected saliva sample was incubated anaerobically in an incubator at 370C for 48 hours. After 48 hours the colonies were identified with their appearance of short, purple chains in the agar. They were counted by the Microbiologist and it was recorded as CFU/ml. This research was done for a period of 6 weeks. Salivary samples, PI, GI, DMFT&DMFS were recorded before the start of the study and the Study Participants were instructed to use 15ml of mouth rinse in the provided measuring cup with 1: 1 dilution. Partici pants were advised to swish it in the mouth for 30 seconds twice daily after tooth brushing for 6 weeks. The participants were instructed to spit the mouth rinse and advised not to swallow. At the end of third week Salivary samples, PI, GI, DMFT&DMFS were recorded and the same procedure was repeated at the end of 6 weeks.

Results

The Data collected regarding all the selected cases were recorded in a Master Chart. Data analysis was done with the help of computer using Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, version 22.0 for Windows).

Using this software frequencies and percentages were calculated for qualitative variables. Means and standard deviations were calculated for quantitative variables. 't' value, chi square and 'p' values were also calculated. Students'' test and ANOVA were used to test the significance. A 'p' value less than 0.05 denotes significant relationship.

Inter group comparison of mutans streptococci values between the three groups at Baseline and after intervention (TABLE 1 AND GRAPH 1)

Data analysis for the mean CFUS of mutans streptococci in between different groups at baseline and after the intervention at third week and sixth week. When comparing the mean CFUS of mutans streptococci at baseline in between each group, group A, B and C (p = 0.993), group A and B (p = 0.922), group A and C (p = 0.912) and group B and C (p = 0.992), the differences noted were not statistically significant ($p > 0.05$).

When comparing the mean percentage reduction in mutans streptococci, CFUs at third week in between each experimental group (A, B and C) the differences noted were all statistically significant ($p < 0.001$).

When comparing the mean percentage reduction in mutans streptococci, CFUs at six week in between experimental group B and C the difference noted was statistically significant (p< 0.001).

Table 1: inter group comparison of mutans streptococci values between the three groups at baseline and after intervention.

Group	*mutans* streptococci values (in millions)					
	Baseline		3rd week		6th week	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Chlorhexidine Group A	10.92	1.16	5.35	0.71	-	-
Cinnamon Group B	10.89	1.16	9.24	1.01	7.55	0.86
Neem Group C	10.89	1.12	7.76	0.77	5.54	0.7
'p' value between Groups						
Chlorhexidine ,Cinnamon	0.993		< 0.001 *		-	
& Neem	0.922		< 0.001*		-	
Chlorhexidine &	0.912		< 0.001*		-	
Cinnamon	0.992		< 0.001*		< 0.001*	
Chlorhexidine & Neem						
Cinnamon & Neem						

Paired t test Anova p>0.05, *p<0.05,

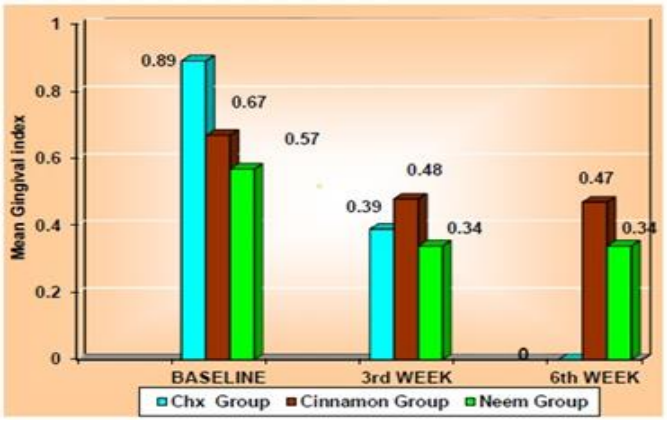
*Statistically significant

Inter group comparison of Gingival Index values between the three groups at Baseline and after intervention (GRAPH 2)

Data analysis for the mean Gingival Index values in between three groups at baseline and after the intervention at third week and sixth week. When comparing the mean Gingival Index values at baseline in between each group, group A, B and C (p < 0.001), group A and B (p < 0.001) and group A and C (p < 0.001) the differences noted were statistically significant.

Expect for comparing the experimental Group B and C the differences noted were not statistically significant (p= 0.068). When comparing the mean percentage reduction in Gingival Index values at third week in between each experimental group (A, B and C) the differences noted were statistically significant for group A, B and C (p =0.034) and group B and C (p = 0.008) The difference noted were not statistically significant for group A and B (p =0.127) and group A and C (p = 0.353). When comparing the mean percentage reduction in Gingival Index values at six week in between experimental group B and C the difference noted was statistically significant (p= 0.012).

Graph 1: Inter group comparison of gingival index values between the three groups at baseline and after intervention.

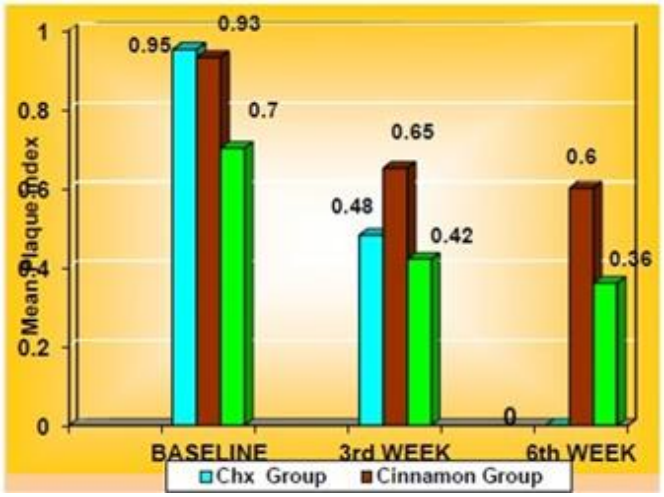


Inter group comparison of Plaque Index values between the three groups at Baseline and after intervention (GRAPH 3)

Data analysis for the mean Plaque Index values in between three groups at baseline and after the intervention at third mean week and sixth week. When comparing the Plaque Index values at baseline in between each groups, group A, B and C (p< 0.001), group A and C (p < 0.001) and group B and C (p < 0.001) the differences noted were statistically significant. When comparing the mean percentage reduction in Plaque Index values at third week in between each experimental group (A,B and C) the differences noted were statistically significant for group A, B and C (p < 0.001) group A and B (p < 0.001) and group A and C (p < 0.001) The difference noted were not statistically significant for group A and C (p =0.341)

When comparing the mean percentage reduction in Plaque Index values at six week in between experimental group B and C the difference noted was statistically significant (p =0.001)

Graph 2: inter group comparison of plaque index values between the three groups at baseline and after intervention.



Discussion

In spite of the tremendous progress in the development of medical sciences, plants continue to be an important source of drugs in many countries across the globe. During past couple of decades reliability and usage of herbal product has become of increasing importance, due to the side effects and complications of many chemical and synthetic medicines. Even though Chlorhexidine was found to be more effective on aerobic and anaerobic microorganisms, well documented side effects of Chlorhexidine like tooth staining, taste alteration, and development of resistance, limits its use especially among children.[4]Therefore, herbal mouth rinse can be considered as a potential plaque inhibitor with special health care needs.[5]

The current study has attempted to show the efficacy of neem and cinnamon as an antimicrobial agent in comparison to the gold standard of mouthwash chlorhexidine. Awareness about the side effects of chemical plaque control agents have brought to light several herbal products that have antibacterial properties. Azadirachta indica extract contains isoprenoids such as nimbin, nimbi in and nimbi din which exert an antibacterial effect against several oral streptococci [(6,7)] and reduces bacterial adhesion to tooth surface.[8] Cinnamon oil has the most potential bactericidal properties. The antibacterial activity of cinnamon oil has been demonstrated, and it has been shown that cinnamon oil alone or in combination with triclosan, gentamicin, or chlorhexidine can effectively inhibit biofilm formation, detach existing biofilms, and kill bacteria in biofilms. Another advantage of the essential oil over antibiotics may be that bacteria do not develop resistance to essential oils.[9]

Available literature shows that there are few studies comparing the efficacy of Chlorhexidine with neem and cinnamon as an antimicrobial agent. The present research is a randomized, parallel arm, double blind controlled trial designed to compare the efficacy of neem and cinnamon as an antimicrobial agent in comparison to the gold standard of mouthwash namely chlorhexidine. To our knowledge this was the first study attempted to compare the efficacy between neem, cinnamon and chlorhexidine both clinically through GI and PI and microbiologically by salivary mutans streptococci count, therefore attempt has been made to make valid comparisons of selected results wherever possible,

The previous research conducted by Kaushal S (2013) to prove the efficacy of Azadirachta indica (Neem) as an antigingivitic agent. The result of this study showed that Azadirachta indica is a very promising anti-gingivitis agent. It can be used as routine adjunct against gingivitis.[10] which is in consistence with the present study.

The previous invitro studies conducted by Aneja rai Kamal et al (2009)[11], Prabuseeni Vasan et al in 2006 and Kalemba et al in 200343 proved that cinnamon oil was found to be a potent antimicrobial agent and showed significant reduction in salivary mutans streptococci[12] this is in concurrence with the present study but this study stands out that it is an Invivo study which used cinnamon as mouth rinse.

In this study, after rinsing with the respective mouthwashes, statistically significant differences were found among neem, chlorhexidine, and cinnamon at 3 weeks and at 6 weeks. Neem, cinnamon and chlorhexidine showed difference in the mean plaque and gingival index scores at these time intervals. This implies that neem, cinnamon was equally effective in inhibiting plaque. The present study indicates that when compared with baseline, all the three mouthwashes showed statistically significant decrease in the plaque index, gingival index and mutans streptococci at 3rd week and 6th week respectively but when compared with herbal mouth washes, Chlorhexidine is superior in reducing the plaque. The results of this study indicate that neem and cinnamon herbal mouth rinses improved the oral hygiene and also reduced the salivary mutans streptococci at 3rd week and 6th weeks when compared to baseline. The results found a significant reduction of salivary *mutans* streptococci in neem and cinnamon herbal mouthwash over a period of three weeks as compared to chlorhexidine. The results of the current study have shown that the mean scores of plaque index, gingival index and colony forming units (CFUs) recorded at baseline and at third week was consistently lower in the group A (chlorhexidine) with the significant difference from the value of group B(Cinnamon) and group C (Neem).

Conclusion

5% neem and 10% cinnamon was considerably efficacious in reducing the *mutans* streptococci count, plaque and gingivitis significantly. 5% neem 10% cinnamon was equally efficacious in reducing the mutans streptococci count, plaque and gingivitis. It proves to be a promising anticariogenic and antiplaque mouth rinse and can be used as a cost effective and safe alternatives to Chlorhexidine.

Limitations of the study

The age group for the present study is narrow and the study results can only be extrapolated to this particular age group and this research included only two salivary samples collected after 3rd week and 6th week followed by the intervention. Therefore, long term or substantivity of the neem and cinnamon mouth rinse could not be compared with that of Chlorhexidine. Further long-term studies are recommended.

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