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RESEARCH ARTICLE

COMPARISON OF CHLORHEXIDINE MOUTHRINSE VERSUS CACAO BEAN HUSK EXTRACT MOUTHRINSE WITH THE ADDITION OF XYLITOL, AS ANTI-PLAQUE AGENTS IN CHILDREN

***Dr. Siya Dukle, Dr. Alok Patel, Dr. Laxmi Lakade, Dr. Preetam Shah, Dr. Rahul Lodaya and Dr. Chetan Bhat**

Department of Pedodontics and Preventive Dentistry, Bharati Vidyapeeth Deemed University Dental College and Hospital, Pune

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ABSTRACT

The purpose of this study was to compare the efficacy of Cacao bean husk extract mouthrinse with the addition of xylitol, with chlorhexidine mouthrinse as antiplaque agents in children. Forty children between 8-12 years of age were chosen from a residential school and randomly allocated into two groups. Group A was given 0.1% Cacao Bean Husk Extract Mouthrinse to be used twice daily for 1 month while Group B was given 0.12% Chlorhexidine Mouthrinse to be used twice daily for 1 month. This study confirms that Cacao Bean Husk Extract mouthrinse with the addition of xylitol, was equally effective as the chlorhexidine mouthrinse in reducing the Strep Mutans counts in saliva and reducing the plaque scores in children.

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INTRODUCTION

Environmental awareness and accountability are rapidly moving to the forefront of humanity's consciousness. Historically, natural products have been used since ancient times and in the folklore for treatment of many diseases and illnesses. Traditional plants and natural phytochemicals can treat dental infections and are considered as a good alternative to these synthetic chemicals. Various products such as turmeric, camphor, clove oil are sought to relieve pain caused due to odontogenic infections, whereas ginger, neem, tulsi, amla, triphala and aloevera, are proven to reduce Streptococcus Mutans counts when used as mouthrinses. Green tea extract, propolis, coffee beans are also under extensive research owing to their high antimicrobial efficacy against various virulent mirobes residing in the oral cavity. Primary causative agent of dental caries and plaque formation in humans is Streptococcus Mutans. It is an anaerobic bacterium known to produce lactic acid as part of its metabolism and produces 3 types of glucosyl transferase (GTFB,GTFC,GTFD) while synthesizing an adherent and water insoluble glucan from sucrose by their cooperative action, which causes the organisms to adhere firmly to the tooth surface, thus causing localized decalcification of the enamel surface. (Osawa *et al*., 2001)

Corresponding author:* **Siya Dukle,
Department of Pedodontics and Preventive Dentistry, Bharati Vidyapeeth Deemed University Dental College and Hospital, Pune

Plaque control by mechanical means including tooth brushing, flossing are most common. However despite its important role in the removal of plaque, it is not practised properly by most individuals and especially children. Factors such as dexterity and motivation, can limit the effectiveness of daily self performed oral hygiene. This difficulty in maintaining adequate plaque control necessitates the use of chemotherapeutic agents. (Fine, 2000) Among the chemotherapeutic agents chlorhexidine is the "gold-standard" or positive control for comparison with other substances due to its proven efficiency (Pires *et al*., 2007). However, the incidence of side effects such as undesirable tooth discoloration, unpleasant taste, dryness and burning sensation in the mouth discourage patients to use this mouthwash. (Saheli P, Momeni Danaie Sh, 2006) Chocolate mouth rinse, 0.1 % (Cacao bean husk extract mouth rinse) is highly effective in reducing streptococcus mutans and plaque accumulation when used as mouth rinse by children. (Percival *et al*., 2006) Study conducted on Cacao bean husk extract by K.Osawa *et al*l in the year of 2000 showed a reduction in colony forming units of Streptococcus mutans by 77% and inhibition of Glucan synthesis by Glycosyl transferase by 57%. (Osawa *et al*., 2001) Xylitol has proven to be an essential substitute to routine sugars, as an overwhelming majority of studies showed a protective effect of xylitol towards tooth decay. It not only helps in maintaining the pH of saliva nd plaque but also reduction of oral microflora, hence offering to be a potent tool that can leave a significant impact.

(Kiet A. Ly *et al*., 2006) Thus considering the side effects of Chlorhexidine mouth rinse and promising effects of Chocolate mouth rinse and Xylitol this study was designed to compare the effectiveness of both the rinses as antiplaque agents in children. Moreover children would be more attracted by the name and flavour of this chocolate mouth rinse, so that regular use could be contemplated.

MATERIALS AND METHODS

Forty Children of both sexes aged between 8-12 years attending a residential school (Shrimati Jijamata Bhausaheb Bhagwat Vidyalaya, Daund-Maharashtra) were randomly chosen for this comparative study of one month duration.

Inclusion criteria

- Patients with age group of 8-12 years
- Patients having positive / definitive positive Frankel's behaviour.
- Patients having dmft = 0 or less than 6.

Exclusion criteria

- Patient with any systemic disorder.
- Patients taking antibiotics since last 3 months or received any periodontal therapy within past 6 months.
- Patients undergoing any surgical procedures.

Subjects were included in the study only after the parents/guardians read the subject information sheet and signed the consent form. The design of this study, the consent forms and the mouthrinse preparation were reviewed and approved by the ethical committee.

Preparation of cacao bean husk extract mouthrinse with xylitol

The ground husks of the cocoa beans (1.0 kg), a by-product of cocoa manufacture, was obtained from the CAMPCO Factory in Puttur, Karnataka. Cocoa bean husks were first treated with 5 g of cellulose, in 4.75 l of distilled water at 50°C for 4 h. Ethanol was then added up to 50% (v/v final concentration) and the mixture was refluxed for 1 h. After filtration, the ethanol was removed by evaporation and the aqueous solution lyophilized to produce a powder. This process yielded 120 gm of powdered extract. The powder was dissolved in distilled water to obtain a mouth rinse with a final concentration of 1 mg/ml in 0.1%. Xylitol was added as per taste to act as a masking agent for the bitterness of the mouthrinse. Preparation of the mouthrinse was carried out at a specialized laboratory. The study subjects were randomly divided into two groups of twenty each, namely Group A and Group B.

Group A: The subjects were given 0.1% 10ml Cacao bean husk extract mouthrinse with the addition of Xylitol to rinse their mouth twice daily for 30 seconds.

Group B: The subjects were given 0.12% 10 ml Chlorhexidine mouth rinse to rinse their mouth twice daily for 30 seconds.

On Day 1, an early morning saliva sample was collected from each study subject. (Fig. 1, Fig. 2) Later, each subject was made to swish the plaque disclosing agent in his mouth, (Fig. 3). After assessment of the index, the subjects were asked to brush their teeth and later use the mouthrinse after a time interval of 30 minutes. Later, the subjects were shown a live demonstration on how to use the mouthrinse and the use was closely monitored. Collection of the saliva sample and determination of plaque index was carried out on Days 7 and Day 30 in a similar fashion. The children were given sterile bottles containing Thioglycollate transport media where in approximately 1ml of saliva was collected and the samples were then transported to the laboratory and processed on the same day. The sample was innoculated on dry Mitis Salivarius Agar with potassium tillurite medium and bacitracin. The plates were incubated at 37 degree celsius for 48 hrs after which the colony characteristics were studied, and the number of colony forming units of MS (CFU/ml) were determined. The baseline and the post treatment Streptococcus mutans counts in saliva and the plaque index in the study of both groups was compared and the data obtained was compiled, analyzed and tabulated statistically.

RESULTS

Results of the microbial analysis (Table 1, Graph 1) revealed that Chlorhexidine mouthrinse showed a reduction in the Strep Mutans count from 1st day to the 7th day and was found to be statistically significant ($p<0.001$). However the decrease of the Strep Mutans counts from saliva of the subjects from Day 7 to day 30 did not show a statistically significant difference. Cacao Bean Husk Extract mouthrinse with the addition of xylitol showed a reduction in the Strep Mutans count from 1st day to the 7th day and was found to be statistically significant ($p<0.001$). (Table 2, Graph 2) However the decrease of the Strep Mutans counts from saliva of the subjects from Day 7 to day 30 did not show a statistically significant difference. Decrease in the Plaque index scores (Table 3,4 Graph 3,4) for both, 0.12% Chlorhexidine as well as 0.1% Cacao Bean Husk Extract mouthrinse showed a statistically significant difference ($p<0.001$), from Day 1 to Day 7 and to Day 30.

Fig.1. Saliva sample collected from each study subject

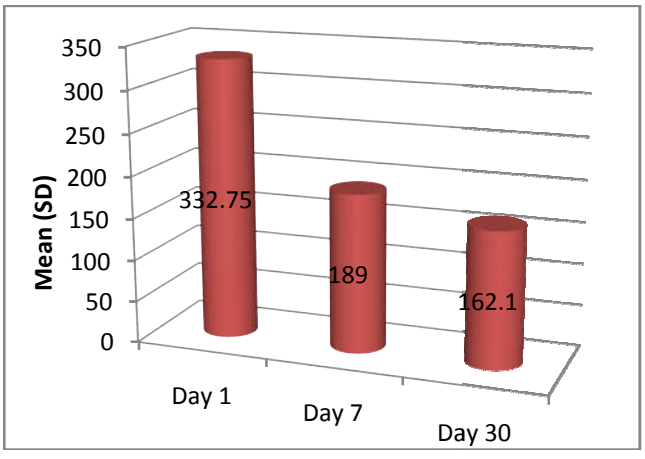
Fig.2. Saliva Samples collected in Sterile containers on Day 1

Fig.3. Plaque disclosing tablet

Table 1. Comparison of colony counts (CFU/ml x 100) at different time intervals in Group B using ANOVA test

Time interval	No of samples	Mean (SD)
Day 1	20	332.75 (78.3)
Day 7	20	189.0 (46.7)
Day 30	20	162.10 (41.9)
F value	-	50.087
P value	-	<0.001**

(p< 0.05 - Significant*, p < 0.001 - Highly significant**)

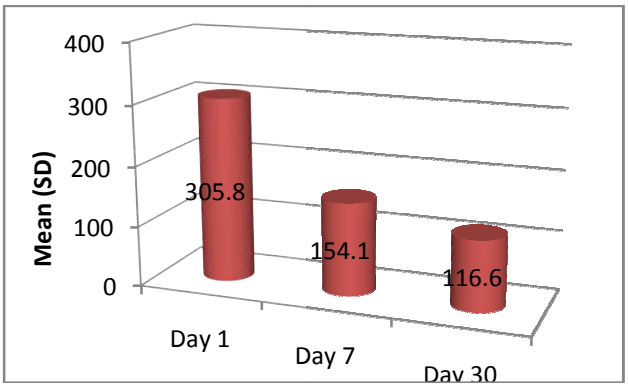


Graph 1. Comparison of colony counts (CFU/ml x 100) at different time intervals in Group B using ANOVA test

Table 2. Comparison of colony counts (CFU/ml x 100) at different time intervals in Group A using ANOVA test

Time interval	No of samples	Mean (SD)
Day 1	20	305.80 (106.8)
Day 7	20	154.10 (54.7)
Day 30	20	116.60 (41.2)
F value	-	37.351
P value	-	<0.001**

(p< 0.05 - Significant*, p < 0.001 - Highly significant**)



Graph 2. Comparison of colony counts (CFU/ml x 100) at different time intervals in Group A using ANOVA test (Intragroup - Group A)

Table 3. Comparison of plaque index scores at different time intervals in Group A using ANOVA test

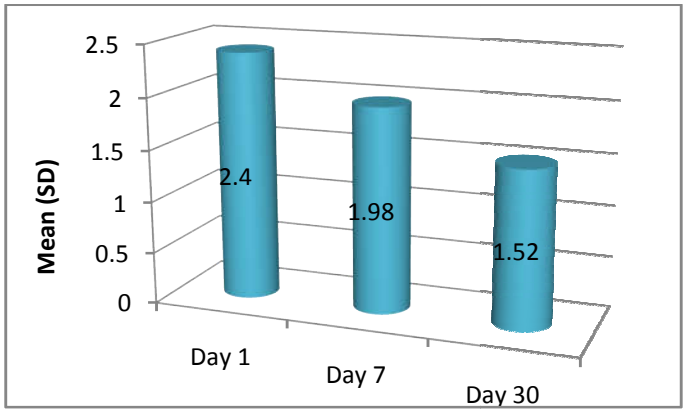
Time interval	No of samples	Mean (SD)
Day 1	20	2.40 (0.3)
Day 7	20	1.98 (0.3)
Day 30	20	1.52 (0.2)
F value	-	35.464
P value	-	<0.001**

(p< 0.05 - Significant*, p < 0.001 - Highly significant**)

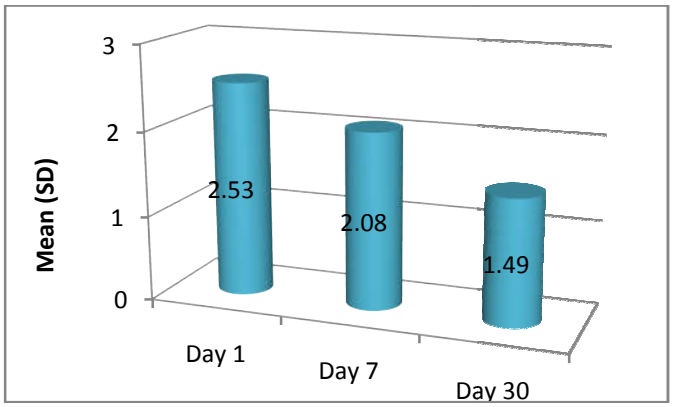
Table 4. Comparison of plaque index scores at different time intervals in Group B using ANOVA test

Time interval	No of samples	Mean (SD)
Day 1	20	2.53 (0.3)
Day 7	20	2.08 (0.3)
Day 30	20	1.49 (0.3)
F value	-	56.384
P value	-	<0.001**

(p < 0.05 - Significant*, p < 0.001 - Highly significant**)



Graph 3. Comparison of plaque index scores at different time intervals in Group A using ANOVA test



Graph 4. Comparison of plaque index scores at different time intervals in Group B using ANOVA test

DISCUSSION

Good oral health is an integral component of good general health. Many children have inadequate oral and general health because of active and uncontrolled dental caries. Treating a carious tooth by reducing the number of cariogenic microorganisms and establishing a favourable oral environment to promote predominant remineralisation of tooth structure over time will stop the caries process. (McDonald and Avery, 2004) Many species of bacteria synthesize glucan polymers and Glucan Binding Proteins (GBP). GBPs include the enzymes that catalyze the synthesis of the glucans, as well as the enzymes capable of hydrolyzing glucans, including starch and cellulose, that can act as substrates for microbial growth. (Banas and Vickerman, 2003) Glucosyltransferase (GTF) is an extracellular or cell-associated enzyme synthesized by the "mutans" group of streptococci as well as the S. sanguis and is responsible for the biosynthesis of extracellular polysaccharides. Glucan formation in dental plaque mediates binding of S. mutans and S. sanguis to one another as well as to other bacteria. Furthermore, GTF has been proven to be an effective antigen in eliciting caries-protective secretory IgA antibodies in rodent models. That led to the identification of GTF as a potential antigen for use in a human caries vaccine. (Hajishengallis and Apostolopoulos, 1989) The role of Streptococcus mutans in cariogenic activity is its ability to adhere to the initially acquired film, produce acids and synthesize insoluble and soluble glucans that help maintain plaque by producing GTFs which metabolize sucrose into free glucans and fructose. (Silvia Barrientos and Adriana Rodríguez, 2011) A variety of compounds capable of controlling dental caries have been extensively surveyed on the basis of the following criteria: antimicrobial activity, inhibition of GTFs by immunological neutralization, enzyme inhibitors, and replacement of sucrose with other sweeteners. However, only limited numbers of compounds from natural products are available because of issues of effectiveness, stability, odour, taste, and economic feasibility. (Paolino and Kashket, 1985)

Plaque control normally means preventive measures aimed at removing dental plaque and preventing it from recurring. This can be accomplished either mechanically, chemically or combined. Arguably the use of a toothbrush and toothpaste is the most commonly employed method of oral hygiene. However, despite its important role in the control of periodontal disease, mechanical plaque control is not properly practiced by most individuals. Therefore, adjunctive use of chemical plaque control was thought to be beneficial. (Addy, 1986) Chlorhexidine, till date is regarded as a Gold standard in chemical plaque control as it possesses various superior properties. It has a wide range of activity against both Gram positive / negative bacteria, and it is also an effective antifungal agent especially against C. albicans. The most important property of this product, is that of antibacterial substantivity, which can last for upto 12 weeks. (Zahed Mohamaddi, 2008)

With all these advantages, chlorhexidine is reported to have a number of local side effects. (Lang and Lindhe, 2006) Brownish discoloration of the teeth, restorative materials and dorsum of the tongue which, may be due to

a) Degradation of chlorhexidine molecule to release parachloraniline,
b) Catalysis of Maillard reaction,
c) Protein denaturation with metal sulfide formation, and
d) Precipitation of anionic dietary chromogens

Taste perturbation where the salt taste appears to be preferentially affected to leave food and drinks with a rather bland taste is also seen. Oral mucosal erosions appear to be an idiosyncratic reaction and concentration dependant and Unilateral or bilateral parotid swellings have also been reported, but is an extremely rare occurrence. Enhanced supragingival calculus formation which is seen may be due to the precipiatation of salivary proteins on the tooth surface Chlorhexidine also has a bitter taste, which is difficult to mask completely. (Lang and Lindhe, 2006) Thus, Chlorhexidine, even though regarded as a Gold standard in chemical plaque control, had several ill effects, and hence there was a need for an alternate product that would overcome the ill effects of Chlorhexidine and be equally effective. Cacao tree is grown in tropics, and Cacao fruit contain 20-40 seeds called Cacao beans. Cacao bean forms the main constituent of chocolate, and ground of Cocoa bean husk is a waste material generated in the chocolate industry (Osawa *et al*., 2001). The cocoa bean husk has been shown to possess cariostatic substances that can inhibit dental caries, however as its taste is bitter. Xylitol is a member of the sugar alcohol or polyol family, which includes other common dietary sweeteners such as sorbitol, mannitol, and maltitol. Xylitol is produced commercially from birch trees and other hardwoods containing xylan. It has been approved by the Food and Drug Administration since the 1960's and is safe to use for children. Since then, it has been commonly used as a sweetener in various food and food products. (Ly *et al*., 2006) In the present study, a comparative evaluation of the efficacy of chlorhexidine mouthrinse with

cacao bean husk extract mouthrinse with the addition of xylitol in children in terms of reduction of Strep Mutans counts in saliva and as anti plaque agents between the two groups was carried out. This Study was carried out at a residential school so that certain factors would remain constant standardizing the study. Firstly, diet was a common factor in all the subjects. As diet plays an important role in the caries process, keeping the diet variable constant in all the subjects benefited the study. Secondly oral hygiene practices of the subjects were similar to each other and as it was a residential school, supervised use of the mouthrinse during the entire study period could also be carried out. This was in conjunction with studies conducted by Rupesh S *et al* in 2014, and J Jasmin Winnier *et al* in 2013, which were also conducted at residential schools. (Winnier *et al*., 2010)

Saliva collection from the study subjects was done in sterile containers, where 1-2 ml of unstimulated saliva was collected. In a study conducted by Mahvash Navazesh in 1982, (Navazesh and Christensen, 1982) it was concluded that unstimulated saliva collected by spitting, was reliable and reproducible for further analysis. As this method was easy and reliable for saliva collected in children, it was selected for our study. Maximum accumulation of plaque takes place within 21 days by the end of 12 weeks well differentiated sub gingival plaque is seen, dominated by Gram negative bacteria. (Newman *et al*., 2006) Hence our study was conducted as a one moth clinical trial, taking samples at Day 1, Day 7 and Day 30. Results of the microbial analysis (Table 1, Graph 1) revealed that Chlorhexidine mouthrinse showed a reduction in the Strep Mutans count from 1st day to the 7th day and was found to be statistically significant ($p<0.001$). This was similar to the study conducted by Sari and Brinci in 2007, Beyth *et al* in 2003 and N.S. Venkatesh Babu *et al* in 2011. However the decrease of the Strep Mutans counts from saliva of the subjects from Day 7 to day 30 did not show a statistically significant difference.

Results of the microbial analysis (Table 2, Graph 2) revealed that Cacao Bean Husk Extract mouthrinse with the addition of xylitol showed a reduction in the Strep Mutans count from 1st day to the 7th day and was found to be statistically significant ($p<0.001$). This was similar to the study conducted by Srikanth *et al* in 2008 and N.S. Venkatesh Babu *et al* in 2011. (Srikanth *et al*., 2008; Venkatesh Babu *et al*., 2011). This decrease in the Strep Mutans counts, may be attributed to the combined mechanism of action of Cacao bean husk, and Xylitol. However the decrease of the Strep Mutans counts from saliva of the subjects from Day 7 to day 30 did not show a statistically significant difference. The statistically insignificant decrease of the Strep Mutans counts for both the mouthrinses from Day 7 to day 30 may be attributed to the fact that the subjects might have shown non compliance while using the mouthrinse, or skipped a day or two when they were not being supervised. The decrease from Day 1 to Day 7 was statistically significant and can be explained by the fact that the subjects who previously had not used a mouthrinse, were interested by the novelty, and practised the use of the mouthrinse strictly under supervision, and slowly lost interest in the same showing non compliance for one or two days. Decrease in the Plaque index scores (Table 3,4 Graph 3,4) for both, 0.12% Chlorhexidine as well as 0.1% Cacao Bean Husk Extract mouthrinse showed a statistically significant difference ($p<0.001$), from Day 1 to Day 7 and to Day 30 which was in accordance with the study conducted by Srikanth *et al* in 2008. (Srikanth *et al*., 2008) The results of this study showed there was no significant difference between both mouth rinses in terms of antimicrobial property, i.e reduction of the Strep Mutans count in saliva and also in the reduction of plaque index scores between the two groups. Thus Chlorhexidine, even though regarded as a Gold standard in chemical plaque control, natural compounds like cacao bean husk, can act in a synergetic manner within the human body and can provide unique therapeutic properties with minimum or no undesirable side effects causing a similar action. Xylitol, a sugar substitute and a cariostatic agent is also a natural compound, derived from birch trees, which when added to this Cacao bean husk extract mouthrinse, enhances its properties, thus making it comparable to the gold standards of Chlorhexidine. However considering the side effects of Chlorhexidine mouthrinse and similar anti microbial and antiplaque properties of Cacao bean Husk Extract mouthrinse with the addition of Xylitol, it is advisable to use this mouthrinse in children.

Conclusion

This study concludes that Cacao Bean Husk extract mouthrinse with the addition of xylitol, may be useful for controlling dental plaque formation and dental caries development in humans. It is a naturally occuring waste product of the chocolate industry which can be recycled and easily prepared into a mouthrinse which has all the beneficial effects. Further studies are required with a larger sample size to establish the results achieved by this study, and towards increasing the efficacy of Cacao bean Husk Extract mouthrinse with the addition of xylitol. However mouthrinses are to be used as an adjunct to mechanical plaque control for better results. This chocolate mouthrinse can be easily prepared and popularised as it is economical and has similar antimicrobial and antiplaque efficiency as that of chlorhexidine mouthrinse, thereby avoiding the side effects of the latter. Moreover children would be more attracted by the name and flavour of the mouthrinse, so regular use can be contemplated.

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