Chocolate mouth rinse: Effect on plaque accumulation and mutans streptococci counts when used by children

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Abstract

Background: Glucosyltransferases (GTF) play an important role in the adherence of bacteria to acquired pellicle. Cocoa bean husk extract (CBHE) has been shown to possess anti-glucosyltransferase and antibacterial activity.

Aim: This study aimed to evaluate the effect of CBHE on plaque accumulation and mutans streptococcus count when used as a mouth rinse by children.

Materials and Methods: Scaling of the teeth of the selected children was done and the children were instructed to refrain from their routine oral hygiene practices till the morning of the fourth day; they were instead given a placebo mouth rinse for use during this period. On the fourth day, saliva was collected from each subject for microbiological analysis and plaque was disclosed and scored using the modified Quigley and Hein plaque index; later, the teeth were cleaned. After 1 week, scaling of the subjects was done and they were given CBHE mouth rinse to rinse their mouth, following the above protocol. The data was statistically analyzed using Wilcoxon’s signed rank test.

Results: There was a 20.9% decrease in mutans streptococci counts and a 49.6% decrease in plaque scores in the CBHE group as compared to the placebo group, which was highly significant (P value < 0.001).

Conclusion: CBHE is highly effective in reducing mutans streptococci counts and plaque deposition when used as a mouth rinse by children.

Keywords: Cocoa bean husk extract, glucosyltransferase, mutans streptococci

Introduction

The purpose of this study was to evaluate the effect of cocoa bean husk extract (CBHE) on plaque accumulation and mutans streptococci counts when used as a mouth rinse by children.

Dental caries tends to remain untreated in many underdeveloped areas, leading to considerable suffering that is often alleviated only by the loss or extraction of the infected tooth. The presence of S mutans has been consistently linked with the etiology of human dental caries.[1]

In their attempts to understand the mechanisms of plaque biofilm development, investigators have discovered several glucan-binding proteins (GBPs). Some of these, the glucosyltransferases (GTFs), catalyze the synthesis of glucan, which is integral to the sucrose-dependent colonization of tooth surfaces by mutans streptococci.[2] The formation of dental plaque leads to localized demineralization due to the accumulation of acids. Theoretically, the inhibition of each step in this process of caries formation contributes to the prevention of dental caries.[3]

The cocoa bean husk is a waste material generated in the chocolate industry. It has been shown to possess two types of cariostatic substances, one showing anti-GTF activity and the other antibacterial activity.[4]

Materials and Methods

This single-blind cross-over study was conducted to evaluate the effect of CBHE on plaque accumulation and mutans streptococci count when used as a mouth rinse by children. The study was conducted in a government-run residential school located in Davangere city after prior approval was obtained from the ethical committee of the Rajiv Gandhi University of Health Sciences.

Selection of subjects

Thirty two children who satisfied the inclusion criteria were randomly selected for the study from among 10-14 year old students of a residential school who had given consent to participate in the study. Children who had received antibiotic medication at any time in the past 3 months were excluded from the study.

Preparation of CBHE

The ground husks of the cocoa beans (1.0 kg), a by-product of cocoa manufacture, was obtained from CAMPCO factory, Puttur, Dakshin Karnataka. Cocoa bean husks were first treated with 5 g of cellulose (Biocatalyst Ltd, UK) 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%.

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**Mouth rinse study**

The selected children underwent scaling of teeth, after which they were given a placebo mouth rinse (0.1% ethanol in distilled water) and advised to use it before and after every meal, between meals, and before going to bed at the nights (a total of nine times per day). Additionally, until the morning of the fourth day, they were instructed to refrain from all their usual oral hygiene practices. The mouth rinse was kept in a container and each subject was given a cup that could contain 100 ml of mouth rinse. Each mouth-rinsing session consisted of five 10-s rinses with 20 ml of mouth rinse. The subjects were asked not to consume tea/coffee and not to eat chocolates during the study period. On the morning of the fourth day, 1.5 ml of unstimulated saliva was collected and plaque was stained with two-tone solution and, later, scored using the modified Quigley and Hein Index.\(^{[6]}\) Only teeth that were fully erupted were scored; partially erupted teeth, fully crowned teeth, and teeth which were mobile were not considered for the scoring. The exclusion criteria for the teeth were similar to the exclusion criteria followed by Garcia-Godoy et al.\(^{[7]}\) in their study done on 6-11 year old children.

After one week, the CBHE mouth rinse was given, instead of the placebo, to the same subjects and they were asked to once again follow the above protocol.

Before giving the subjects the mouth rinse, prophylaxis was done and after collection of saliva and scoring the plaque on the morning of fourth day, the teeth were cleaned using cotton and by brushing.

**Microbiological analysis**\(^{[8]}\)

For the microbiological analysis, 1.5 ml of unstimulated saliva was collected in a bottle after each study period. It was then diluted ten folds with normal saline.

Mitis salivarius (MS) agar was prepared according to the manufacturer’s instructions as follows: 90 g of agar was mixed in 1000 ml of distilled water and the mixture was boiled to ensure complete dissolution; this solution was then autoclaved at 15 lb pressure and 121°C temperature for 15 min. After cooling to 50-55°C, 1 ml of 0.1% potassium tellurite was added to this mixture. This final mixture was poured into petri dishes. Once the MS agar plates were dry, saliva samples were inoculated onto the plates, which were incubated for 48 h at a temperature of 37°C. The colonies were counted using an automatic colony counter. The numbers of colony forming units (CFU) per milliliter was recorded in the pre-structured proforma.

The results were statistically analyzed using Wilcoxon’s signed rank test.

**Results**

The S mutans counts in the placebo group (PL) ranged from 1.42 to 2.93 with a mean value of 2.15 and a standard deviation of \(\pm 0.45\) where as the S mutans counts in cocoa bean husk extract (CBHE) group ranged from 1.17 to 2.49 with a mean value of 1.7 and a standard deviation of \(\pm 0.04\) (all values expressed as a multiple of \(10^6\)) \(\text{[Table 1].}\) When cocoa bean extract (CBHE) group was compared with placebo (PL) group there was a mean difference of 0.45 and a 20.9% reduction in S mutans counts, with a z-value of 4.46 and a highly significant p-value \(< 0.001\). The plaque scores in the placebo (PL) group ranged from 2.52 to 3.78 with a mean score of 2.08 with a mean score of 1.69 and a standard deviation of \(\pm 0.24\) \(\text{[Table 2].}\) When cocoa bean extract (CBHE) group was compared with placebo (PL) group there was a mean difference of 1.66 and a 49.6% reduction in plaque score, with a z-value of 4.46 and a highly significant p-value \(< 0.001\). The difference in the efficacy of both the mouth rinses, with respect to S mutans counts and plaque scores, in each subject can be appreciated from Graphs 1 and 2.
Discussion

S mutans was first described by J.K. Clark in 1924 after he isolated it from a carious lesion but it was not until the 1960s, when researchers began studying dental caries in earnest, that real interest in this microbe was generated. Researchers have identified several hundred genes that appear to be unique to this organism. These are potential drug targets, because disrupting them would disable the pathogen without harming other bacteria in the mouth.[8]

Many species of bacteria synthesize glucan polymers and GBPs. Common forms of glucan include glycogen, a storage form of glucose, and the beta-linked-D-glucans that modulate osmolarity within the periplasm of gram-negative bacteria. 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. For many oral streptococci, glucans comprise an extracellular slime layer produced in the presence of sucrose that promotes adhesion and the formation of a dental plaque biofilm. These glucans are synthesized from sucrose by the enzymatic action of one or more GTFs and can be water-insoluble or soluble.[2]

The synthesis of extracellular glucan is an integral component of the sucrose-dependent colonization of tooth surfaces by species of the mutans streptococci. In their attempts to understand the mechanisms of plaque biofilm development, investigators have discovered several GBPs. Some of these, the GTFs, catalyze the synthesis of glucan, whereas others, designated only as GBPs, have affinities for different forms of glucan and contribute to aspects of the "plaque" biology of their host organisms. The functions of these latter GBPs include dextran-dependent aggregation, dextranase inhibition, plaque cohesion, and perhaps cell wall synthesis. In some instances, their glucan-binding domains share common features, whereas in others the mechanism for glucan binding remains unknown. Recent studies indicate that at least some of the GBPs modulate virulence and some can act as protective immunogens within animal models. Overall, the multiplicity of GBPs and their aforementioned properties are testimonies to their importance. Future studies will greatly advance the understanding of the distribution, function, and regulation of the GBPs and place in perspective the facets of their contributions to the biology of the oral streptococci.[2]

A substantial body of literature supports the importance of GTFs and GBPs in dental caries based on experiments in which immunization with these proteins (or portions of them) can reduce caries rates upon challenge with S mutans. The GTFs have been an obvious target for immunization, given their integral contribution to sucrose-dependent colonization and accumulation.[2]

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. There are at least two major classes of GTFs in common beverages and foods: some monosaccharides or oligosaccharides and the polyphenolic compounds. Fructose; glucose-oligomers, such as maltose, isomaltose, and panose; and structural isomers of sucrose, such as palatinose and trehalulose have been shown to inhibit insoluble glucan synthesis by GTFs from sucrose and also to be cariostatic or noncariogenic in specific pathogen free (SPF) rats infected with mutans streptococci. In addition, several types of polyphenols isolated from plant sources such as tea leaves and betel nuts have been shown to exhibit similar inhibitory activity against GTFs from mutans streptococci. However, only limited numbers of compounds from natural products are available because of issues of effectiveness, stability, odour, taste, and economic feasibility.[9]

Inhibition of GTFs by cocoa, coffee, and tea was partly due to gelatin-perceptible tannins and partly due to components that exhibited properties of monomeric polyphenols; these constituents may play a role in regulating dental plaque formation in vivo and, thereby, may have long-term effects on the development of dental caries.[10]

The cocoa bean husk has been shown to possess two types of cariostatic substances that can inhibit experimental dental caries in rats infected with mutans streptococci: one shows anti-GTF activity and the other antibacterial activity. Chromatographic purification has revealed high-molecular-weight polyphenolic compounds and unsaturated fatty acids as the active components. The former, which showed strong anti-GTF activity, were polymeric epicatechins with C-4 beta and C-8 intermolecular bonds estimated to be 4636 in molecular weight in an acetylated form. The latter, which showed bactericidal activity against S mutans, were determined to be oleic and linoleic acids, and demonstrated
a high level of activity at a concentration of 30 µg/ml. The cariostatic activity of the cocoa bean husk is likely caused by these biologically active constituents.[4]

When a water-soluble extract from cocoa-extracted powder (CEPWS) was added to a cariogenic model food, a white chocolate-like diet that contained 35% sucrose, it was found that CEPWS significantly reduced caries scores in SPF rats infected with Streptococcus sobrinus 6715 as compared to control rats fed a white chocolate-like diet. CEPWS markedly inhibited water-insoluble glucan (WIG) synthesis in vitro through the action of crude GTFs from Streptococcus sobrinus B13N.[11]

When CBHE was examined for its inhibitory effects on the caries-inducing properties of mutans streptococci in vitro and on caries development in SPF Sprague-Dawley rats infected with mutans streptococci, it reduced the growth rate of almost all oral streptococci examined and resulted in the reduction of acid production. Insoluble glucan synthesis by the GTFs from S mutans MT8148R and S sobrinus 6715 was significantly inhibited by CBHE. Hence, the sucrose-dependent cell adherence of mutans streptococci was also depressed by CBHE and administration of CBHE in drinking water resulted in significant reductions of caries development and dental plaque accumulation in rats infected with either S sobrinus 6715 or S mutans MT8148R, and the minimum cariostatic concentration was found to be 1.0 mg/ml.[12]

In the present study, a concentration of 1 mg/ml of CBHE was used as it had been proven to be effective.[5,12] When the CBHE treatment was compared with the placebo treatment, there was a 20.9% reduction in mutans streptococci counts and a 49.6% reduction in plaque score with the former, which was highly significant (P < 0.001). These findings show that CBHE significantly reduced plaque deposition and mutans streptococci counts when used as mouth rinse.[5]

Conclusion

CBHE rinse was highly effective in reducing mutans streptococci counts and plaque accumulation when used as mouth rinse by children. It can feasibly be incorporated in chocolates, chewing gums, mouth rinses, and beverages to prevent dental caries. Though no side effects were observed during the study period, the common complaint expressed by the children was regarding the bitter taste of the rinse. Addition of noncariogenic sugar substitutes should make it more acceptable, especially for children. This study needs to be repeated with a larger study group, in different age-groups, and in different geographical areas.

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References


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