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Chocolate Disinfectant: Effectiveness of Cocoa Bean Husk Extract on *Streptococcus mutans* in Used Toothbrushes

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ABSTRACT

Purpose: The objective was to determine the effectiveness of chocolate as an antimicrobial solution on *Streptococcus mutans* in used tooth brushes. ***Methods:*** Sixty children used their toothbrushes twice a day, for seven consecutive days. The toothbrush bristles were then immersed into the chocolate antimicrobial solution for 12 hours. They were then placed into test tubes containing the resultant suspensions were three-fold diluted. Aliquots of 0.1 ml were plated in Mitis Salivarius Agar (enriched with sucrose) using dilution and plating method. Incubation was done in an anaerobic jar for 72 hours at 37°C. ***Results:*** The results obtained showed that there was 32.25% reduction in the *Streptococcus mutans.* The difference between them was found to be statistically significant ($p<0.001$). ***Conclusion:*** Bacterial contamination of toothbrushes is a major cause of concern. The Chocolate antimicrobial solution consisting of Cocoa Bean Husk used in this study proved to be effective toothbrush decontaminant.

KEYWORDS: Chocolate; Cocoa Bean Husk; Toothbrush; Contamination; Antimicrobial solutions; *Streptococcus mutans*

INTRODUCTION

Streptococcus mutans count outnumbers many other organisms in development of caries, plaque and biofilm. Efficient removal of dental plaque is thus essential for maintaining oral health. In their attempts to understand the mechanisms of plaque biofilm development, investigators have discovered several glucan-binding proteins (GBPs) and the glucosyltransferases (GTFs), catalyze the synthesis of glucan, which is integral to the sucrose-dependent colonization of tooth surfaces by mutans streptococci.[1] The most common oral hygiene aid used to improve the oral health of an individual is toothbrush. They are manufactured free of microorganisms.[2,3] After a single use, within 30 seconds to 4 minutes it gets contaminated by a wide array of bacteria, viruses, yeasts and fungi present both in the oral cavity and also the external environment.[4] These microorganisms remain viable for periods ranging from 24 hours to 7 days. These contaminated toothbrushes might play a role in systemic and oral diseases.[5,6] Studies have investigated the microbial contamination of toothbrush bristles after use with some of the most effective disinfection methods.[2-6] However, there are no studies regarding the effect of cocoa bean husk in reducing toothbrush contamination.The cocoa bean husk (CBH) is a waste material generated in the chocolate industry. Its extract i.e. cocoa bean husk extract (CBHE) has been shown to possess two types of cariostatic substances, one showing anti-GTF activity and the other antibacterial activity.[7,8]

The purposes of this study were to evaluate:

1. The contamination of toothbrushes by *Streptococcus mutans.*
2. To check the efficacy of CBH in the disinfection of these toothbrushes through a clinical trial.

METHODOLOGY

Inclusion Criteria

• Subjects having DMFT score = 0
• Subjects aged 18 to 25 years

Exclusion Criteria

- Subjects who did not give consent.
- Subjects using antibiotic medications, mouthwashes, chewing gums, tobacco at the time of the study or 15 days prior to it.
- Subjects having any oral or systemic disease.
- Subjects undergoing any dental treatment.

Schedule for the Study

The study duration was an experimental trial conducted for 1 week (7 days).

Selection of Study Subjects

A total of 60 dental students aged 18 to 25 years (30 males and 30 females) were selected using simple random sampling from Rajarajeswari Dental College and Hospital, Bengaluru, India. Informed consent was obtained from the participants and ethical clearance from the institution.

Blinding Procedure

At the beginning of a week each participant was given a Colgate toothbrush and toothpaste with the following oral hygiene instructions:-

- Brush twice in a day
- Method: Modified Bass method
- Time required: 2 to 3 minutes
- The toothbrushes should be exclusively used by the participant and not to be shared with anyone
- The toothbrush was to be placed upright in a rack and should be kept isolated.

STUDY PROCEDURE

Sterility Control

Five new toothbrushes which were freshly opened from the packets were subjected to microbial analysis to check for *S. mutans* colonies on bristles. This was done to ensure that the new toothbrushes were free from contamination before its use by study subjects.

Preparation of Test Solutions

Phosphate Buffered Saline (PBS)

Commercially available

Preparation of CBHE[8,9]

The ground husks of the cocoa beans (1.0 kg), a by-product of cocoa manufacture, was obtained from CAMPCO factory, Puttur, Dakshin Karnataka, India. 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%.

MICROBIOLOGICAL ANALYSIS

At the end of 1 week, the toothbrushes were collected in sterile plastic bags. They were sent for further processing to Al-Ameen Microbiology Lab, Bengaluru. Under aseptic conditions, the head of each toothbrush was decapitated and was immersed in pre-sterilized test tubes containing 10 ml PBS having pH 7.4. Totally, 10 tufts of bristles of each toothbrush were cut in aseptic conditions and 2 tufts of those were immersed in solutions for 12 hours. The two solutions were PBS control and CBHE (Fig. 1). All 60 test tubes were subjected to vortexing for 15 seconds using cyclomixer. The resultant suspensions were three-fold diluted. Aliquots of 0.1 ml were plated in Mitis Salivarius Agar (enriched with sucrose) using dilution and plating method. Incubation was done in an anaerobic jar for 72 hours at 37°C. Colonies were counted using colony counter and then expressed as number of CFU/mL of saliva. The actual numbers of colonies were multiplied with 1×10^3 as the samples were diluted 1000 times.

STATISTICAL ANALYSIS

Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on mean ± SD (min-max) and results on categorical measurements are presented in number (%). Significance is assessed at 5% level of significance. Student t-test (paired) has been used as the data is dependent set or measured on different materials on same subjects. The statistical software used was SPSS 15.0.

RESULTS

The results obtained showed that there was 32.25% reduction in the *Streptococcus mutans* in the CBHE group. The difference between them was found to be statistically significant ($p<0.001$).

Groups	Range	Mean± Standard Deviation
PBS Control	25.0-36.0	31.03 ± 3.16
CBHE	19.00-34.00	21.00±2.79

Table 1: Comparison of the *Streptococcus mutans* 10^3 CFU/mL of the antimicrobial agents with control

Comparison	Difference		P value
	Male	Female	
PBS Control Versus CBHE	19.07	21.00	0.019

Table 2: Sexwise comparison of effect of different antimicrobial solutions on *S. mutans* compared with control

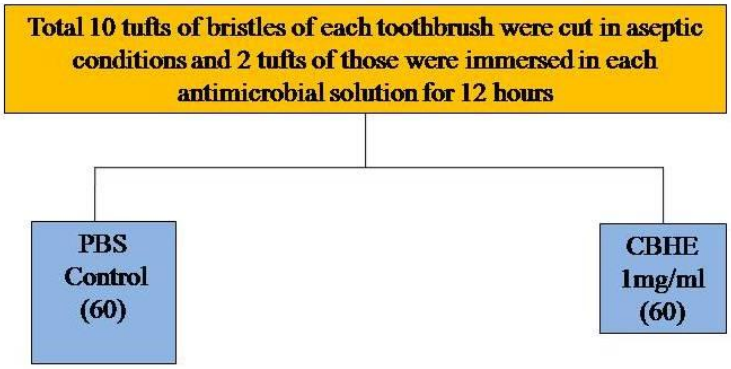

Fig. 1: Antimicrobial Solutions

The mean ± SD observed in control group was 31.03 ±3.16; the mean ± SD observed in CBHE group was 21.00 ±2.79 ($p<0.001$) (Table 1). Sexwise comparison of effect of different antimicrobial solutions on *S. mutans* compared with control was done sex wise ($p<0.019$) (Table 2).

DISCUSSION

Storage conditions of toothbrushes are an important factor for bacterial survival. Plaque biofilm on the old toothbrush bristles were also observed despite the time of use and storage conditions. Time necessary for colonization is contradictory varying from 1 to 30 days.[10] The synthesis of extracellular glucan is an integral component of the sucrose-dependent colonization of tooth surfaces by species of the *Streptococci mutans*. In their attempts to understand the mechanisms of plaque biofilm development, investigators have discovered several GBPs and GTFs which catalyze the synthesis of glucan and contribute to aspects of the "plaque" biology of their host organisms. 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 *Streptococcus mutans*.[11] 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 *Streptococcus mutans*, were determined to be oleic and linoleic acids.[7] 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* 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*.[12] The administration of CBHE in drinking water resulted in significant reductions of caries development and dental plaque accumulation in rats infected with *Streptococcus sobrinus* and *Streptococcus mutans*, and the minimum cariostatic concentration was found to be 1.0 mg/ml. In the present study, a concentration of 1 mg/ml of CBHE was used as it had been proven to be effective.[8,9] When the CBHE treatment was compared with the PBS there was a 32.25% reduction in *Streptococcus mutans* counts which was highly significant ($p < 0.001$). These findings show that the chocolate disinfectant consisting of CBHE can be used as an effective disinfectant against *Streptococcus mutans* in contaminated toothbrushes.

CONCLUSION

Bacterial contamination of toothbrushes is a major cause of concern. The present study revealed the antimicrobial activity of CBHE was highly effective in reducing *Streptococcus mutans* counts in contaminated toothbrushes. Children and adults alike can be motivated to keep their tooth brushes free from contamination in this chocolate disinfectant. A detailed study is required to know the effect of these chemicals on other transmissible microorganisms in contaminated toothbrushes. Establishing an easy and effective method for disinfecting a toothbrush would be an important and economical way to prevent the continuation of oral diseases.

BIBLIOGRAPHY

1. Banas JA, Vickerman MM. Glucan-binding proteins of the oral *Streptococci*. Crit Rev Oral Biol Med. 2003;14:89-9.
2. Bhat PK, Badiyani BK, Sarkar S,Chengappa S, Bhaskar NN. Effectiveness of Antimicrobial Solutions on *Streptococcus mutans* in used Toothbrushes.World J Dent. 2012;3(1):6-10.
3. Balappanavar AY, Nagesh L, Ankola AV, Tangade PS,Kakodkar P, Varun S. Antimicrobial efficacy of various disinfecting solutions in reducing the contamination of the toothbrush: A comparative study. Oral Health Prev Dent. 2009;7(2):137-45.
4. Taji SS, Rogers AH. The microbial contamination of toothbrushes: A pilot study. Aust Dent J Apr. 1998;43(2):128-30.
5. Glass RT, Lare MM. Toothbrush contamination: A potential health risk. Quintessence Int Jan. 1986;17(1):39-42.
6. Nelson-Filho P, Faria G, da Silva RA, Rossi MA, Ito IY. Evaluation of the contamination and disinfection methods of toothbrushes used by 24-to 48-month-old children. J Dent Child. 2006;73(3):152-58.
7. Osawa K, Miyazaki K, Shimura S, Okuda J, Matsumoto M, Ooshima T. Identification of cariostatic substances in the cacao bean husk: Their anti-glucosyltransferase and antibacterial activities. J Dent Res. 2001;80:2000-4.
8. Srikanth RK, Shashikiran ND, Subba Reddy VV. Chocolate mouth rinse: Effect on plaque accumulation and *mutans Streptococci*counts when used by children. J Indian Soc Pedod Prev Dent. 2008;26(2):67-70.
9. Matsumoto M, Tsuji M, Okuda J, Sasaki H, Nakano K, Osawa K, *et al.* Inhibitory effects of cocoa bean husk extract on plaque formation *in vitro* and *in vivo* . Eur J Oral Sci. 2004;112:249-52.
10. Nelson Filho P, Macari S, Faria G, Assed S, Ito IY. Microbial contamination of toothbrushes and their decontamination. Pediatr Dent. 2000;22(5):381-84.
11. Banas JA, Vickerman MM. Glucan-binding proteins of the oral *Streptococci*. Crit Rev Oral Biol Med. 2003;14:89-9.
12. Kyoko Ito, Yuko Nakamura, Takahisa Tokunaga, Daisuke Iijima, Kazuo Fukushima. Anti-cariogenic properties of a water-soluble extract from cacao. Biofactors. 2005;23:141-50.

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