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Effect of theobromine in antimicrobial activity: An *in vitro* study

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ABSTRACT

Background: This study aims to compare the antimicrobial activity of theobromine and two commercially available kid's fluoride toothpastes.

Materials and Methods: *In this in vitro* study a chocolate-based nonfluoride toothpaste, Theobromine (Group A) and two commercially available kid's fluoride toothpaste, Kidodent (Group B), Colgate kids toothpaste (Group C) were used in this study. Freeze-dried stock culture of the strains of *Streptococcus mutans*, *Lactobacillus acidophilus*, *Enterococcus faecalis*, and *Candida albicans* was cultured in their respective selective media. Dentifrices were added to the wells punched out in the culture media and incubated. The zones of inhibition were measured to find out the antimicrobial activity. all data statistically analyzed SPSS using Kolmogorov–Smironov and Shapiro–Wilk's tests. one-way ANOVA was done and Turkey's honest significant difference *post hoc* test was done for pairwise comparison $P \leq 0.001$.

Results: Theobromine showed a greater zone of inhibition, which was statistically significant when compared to other two kid's fluoride toothpastes.

Conclusion: Theobromine shows more antimicrobial effect against *S. mutans, L. acidophilus*, and *E. faecalis*, whereas almost a neutral effect with *C. albicans*. Theobromine though nonfluoridated toothpaste showed greater zones of inhibition than other commercially available fluoridated kid's toothpastes.

Key Words: Antimicrobial, microorganisms, theobromine, toothpastes

INTRODUCTION

Tooth decay occurs when bacteria in the mouth turn sugar into acids, which eat away at the tooth's surface and cause cavities.[1] Compounds in the cocoa bean husk have an antibacterial effect and also fight against plaque. This makes chocolate less harmful than many other sweet foods your dentist might warn you against because the antibacterial agents in cocoa beans offset its high sugar levels.

The key to cocoa's dental benefits, according to Sadeghpour, is a substance called theobromine. A water-insoluble, crystalline bitter powder, theobromine is an alkaloid of the cacao plant and is therefore found in chocolate along with teas and other foods. Theobromine helps harden tooth enamel, making teeth less susceptible to decay.[2]

Theobromine is one such major constituent in cocoa bean and found in high concentration in dark than in milk chocolate. Since plaque control forms the basis for caries prevention, the type of toothpaste and its constituents becomes more important. The success of any toothpaste in part lies on its ability

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to eliminate pathogenic oral microflora. Toothpaste containing fluoride had been shown to exhibit antimicrobial activity.[3] Similarly, some studies have shown that cocoa bean husk extract is highly effective in reducing mutans streptococci counts and plaque deposition when used as a mouthrinse by children.[4] Furthermore, the chocolate antimicrobial solution consisting of cocoa bean husk was proved to be effective toothbrush decontaminant.[5] However, there are no prior studies regarding the antimicrobial efficacy of cocoa bean husk (theobromine) comparing fluoride toothpastes and nonfluoride kids toothpaste.

Hence, the purpose of this pilot study was to evaluate the antimicrobial activity of nonfluoridated Theobromine paste.

MATERIALS AND METHODS

In this *in vitro* study. To demonstrate antimicrobial activity, utilized a 2%–4% of theobromine (Group A). Moreover, other two commercially available kid's toothpastes, 495 ppm of Kidodent toothpaste (Group B) and 500 ppm of Colgate kid's toothpaste (Group C).

Freeze-dried stock culture of the reference strains of *Streptococcus mutans*, *Lactobacillus acidophilus*, *Enterococcus faecalis*, *and Candida albicans* was used for the study. The organisms were cultured in Trypticase soy broth and transferred to the selective media to revive from the stock. All the microorganisms such as *S. mutans*, *L. acidophilus*, *E. faecalis*, *and C. albicans* were cultured in Mueller–Hinton agar.

The purity of each test stain was checked during each trial using subculture, Gram stain, and colony morphology. Antimicrobial susceptibility was checked using the ditch method.

The Mueller–Hinton's agar was used to demonstrate the antimicrobial effect on aerobes.[3] Three wells were prepared (4 mm in diameter and 3 mm deep) using a sterile metallic template. The agar plates were streaked with the each stock culture microorganisms. Each stock culture contains *S. mutans*, *L. acidophilus*, *E. faecalis*, and *C. albicans.* Using a sterile spoon excavator, 20 g of Theobromine (2%–4%) toothpastes was dispersed into the first well, 20 g of Kidodent toothpastes (495 ppm) was dispersed into the second well, and 20 g of Colgate kid's toothpastes (500 ppm) is dispersed into the third well. These plates were incubated at 37°C for 48 h. After incubation, zones of inhibition were examined, which appeared as a clear, circular halo surrounding the wells that contained the dentifrice [Figure 1]. Diameters of the zone of inhibition were measured with Hi Antibiotic Zone Scale. The mean diameter of the zone of inhibition (in mm) represented the inhibition value of the tested product. The test was repeated six times in triplicate to overcome any technical errors that might have occurred during a single attempt. Measurements were tabulated and subjected to statistical analysis to know the significance.[3]

RESULTS

The results showed that the toothpaste containing theobromine showed greater zone of inhibition that indicates more effective when compared to other two kids toothpastes for all the three tested microorganisms.

Based on the measurement scores, all data statistically analyzed (SPSS Inc. 21.0, Chicago IBM) using Kolmogorov–Smironov and Shapiro–Wilk's tests were done to check the normality of the zone of inhibition. Results showed that the zone of inhibition in *S. mutans* followed normal distribution, and hence, to compare the mean zone of inhibition between toothpastes, one-way ANOVA was done and Turkey's honest significant difference *post hoc* test was done for pairwise comparison.

On the other hand, the zone of inhibition in *L. acidophilus* and *E. faecalis* did not follow a normal distribution, and hence, Kruskal–Wallis test was done to compare mean zone of inhibition between toothpastes and Bonferroni-corrected Mann–Whitney test was done for pairwise comparison.

Theobromine (Group A) showed greater zones of inhibition which is higher antimicrobial activity against

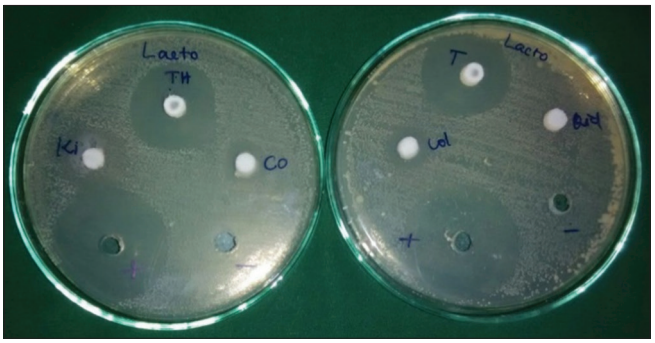

Figure 1: Zone of inhibition observed in *Lactobacillus acidophilus* for toothpastes using Muller–Hinton agar.

S. mutans when compared to Kidodent (Group B) and Colgate kids toothpaste (Group C) which was statistically significant, [Table 1] while comparing Group A with both Group B and C, Group A shows greater zones of inhibition which was statistically significant [Figure 2]. Whereas, when comparing Group B and Group C shows equal zones of inhibition which was not statistically significant [Table 2].

Table 3 summarizes that the theobromine shows higher zones of inhibition in *L. acidophilus* when compared to Kidodent (Group B) and Colgate kid's toothpaste (Group C) which was statistically significant [Figure 1].

Table 4 summarizes that the theobromine shows higher zones of inhibition in *E. faecalis* when compared to Kidodent (Group B) and Colgate kid's toothpaste (Group C) which was statistically significant.

Table 5 summarizes that the theobromine (Group A) shows no zones of inhibition in *C. albicans* when compared to Kidodent (Group B) and Colgate kid's toothpaste (Group C) which was statistically significant [Figure 3]. Table 6 summarizes that the zone of inhibition observed in Group A was higher when compared to Group B in *L. acidophilus* which was statistically significant. When comparing to Group A and Group C, Group C shows least antimicrobial effect which was not statistically significant. Similarly, when comparing to Group B and Group C, Group B shows least antimicrobial effect which was not statistically significant. Similarly, the zone of inhibition observed in Group A was higher in *E. faecalis* when compared to B which is not statistically significant. When comparing to Group A and Group C, Group C shows least zones of inhibition, while comparing to Group A which was statistically significant. When comparing to Group B and Group C, Group B shows least antimicrobial effect which was not statistically significant.

Table 1: One-way ANOVA to compare mean zone of inhibition values (mm) between different toothpastes against *Streptococcus mutans*

Group	*n*	Mean	SD	*F*	*P*
Group A	6	16.83	1.329	61.118	<0.001
Group B	6	8.33	1.751		
Group C	6	7.17	1.835		
Total	18	10.78	4.697		

SD: Standard deviation

Table 2: Tukey's honestly significant difference *post hoc* tests for multiple comparisons between different toothpastes against *Streptococcus mutans*

Group	Mean difference	*P*
Group A		
Group B	8.500	<0.001
Group C	9.667	<0.001
Group B		
Group C	1.167	0.459

Table 3: Kruskal-Wallis test to compare zone of inhibition between different toothpastes against *Lactobacillus acidophilus*

Organism	Toothpaste	*n*	Mean rank	*P*
Lactobacillus	Group A	6	15.50	0.001
	Group B	6	4.00	
	Group C	6	9.00	

Table 4: Kruskal-Wallis test to compare zone of inhibition between different toothpastes against *Enterococcus faecalis*

Organisms	Toothpaste	*n*	Mean rank	*P*
Enterococcus faecalis	Group A	6	3.92	0.001
	Group B	6	9.17	
	Group C	6	15.42	

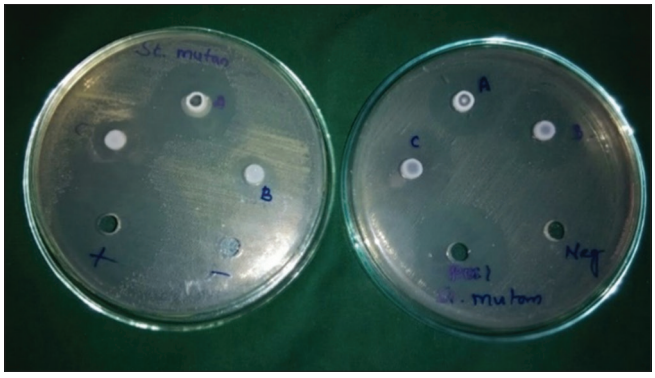

Figure 2: Zone of inhibition observed in *Streptococcus mutans* for toothpastes using Muller– Hinton agar.

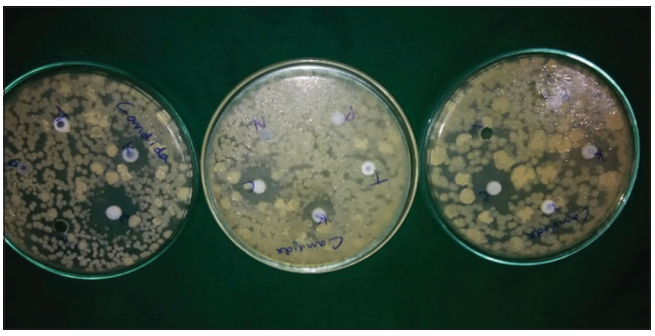

Figure 3: Zone of inhibition observed in *Candida albicans* for toothpastes using Muller– Hinton agar.

Table 5: Kruskal-Wallis test to compare zone of inhibition between different toothpastes against *Candida albicans*

Organism	Toothpaste	*n*	Mean rank	*P*
Candida albicans	Group A	6	16.50	0.001
	Group B	6	3.00	
	Group C	6	8.00	

Table 6: Bonferroni adjusted Mann-Whitney test for pairwise comparison of toothpastes against *Lactobacillus* and *Enterococcus faecalis*

Pair	*P*	
	Lactobacillus	*Enterococcus faecalis*
Group A versus Group B	<0.001	0.228
Group A versus Group C	0.085	<0.001
Group B versus Group C	0.275	0.104

DISCUSSION

Diet and change in food habits from traditional food to junk food also plays a major role in the initiation of dental caries. Chocolate, being the king of junk foods, a common misconception is that it is one of the main culprits for causing dental caries. However, the truth being, among all cariogenic substance, chocolate does not top the list. Furthermore, cocoa butter within the chocolate is said to protect the teeth, giving it a coat of buttery goodness that can resist damage from sugar. Second, there is an aspect of the cocoa plant that could actually prevent cavities and tooth decay.[4] While we do not see the husk of the cocoa bean, it is there when cocoa is in its natural state. Furthermore, the good aspect of chocolate which is derived from cocoa bean is that the cocoa bean contains substances which are anticariogenic. The cocoa bean husk is a waste material in the chocolate industry known to contain a large amount of polyphenols and dietary fibers such as cellulose, pectin, and lenin.[5]

Cocoa bean husk is separated from the preroasted beans of *Theobroma cacaa*. The cocoa bean husk has been shown to possess two types of cariostatic substances, one showing anti-glucosyltransferase (GTF) activity and the other antibacterial activity.[6] Theobromine is one of the major constituents in the cocoa bean. Theobromine (theobromide), which was previously known as xantheose, is a bitter alkaloid of the cocoa plant. It is also found in the leaves of the tea plant and the kola (or cola) nut. It is in the methylxanthine class of chemical compound which also includes the similar compounds such as theophylline and caffeine. Cocoa bean naturally contains 1%–4% theobromine. The amount of the theobromine in cocoa powder ranges from 1.2% to 2.4% higher concentrations of theobromine is found in dark than in milk chocolate.[7] In our present study, 2%–4% concentration of theobromine was used along with sorbitol, xylitol, glycerin, xanthan gum, sodium benzoate, titanium dioxide, calcium acetate, and sodium hydrogen phosphate.

The organisms employed *S. mutans* microorganism produces three types of GTF (GTFB, GTFC, and GTFD), and synthesize an adherent and water-insoluble glycan from sucrose, which causes the organisms to adhere firmly to the tooth surface. The adherent glycan also contributes to the formation of dental plaque, in which the accumulation of acids leads to localized decalcification of the enamel surface. Cocoa bean husk extract is said to contain higher molecular weight polyphenolic compound which is said to have strong anti-glycosyltranseferase property. Apart from this, it contains unsaturated free fatty acids such as oleic and linoleic acids, which exhibit antibacterial activity against *S. mutans*. The cariostatic property of the cocoa bean husk is due to these biologically active constituents.[6] Based on composition, majority of the antimicrobial effects of commercially available kid's toothpastes are attributed to fluoride content, in the form of sodium monofluorophosphate (concentration of 495 ppm). The theobromine used in the present study has no added fluoride content, and it showed statistically significant antimicrobial efficacy when compared to other fluoridated kids toothpaste.

The anticariogenic effect of theobromine by examining its ability to cause remineralization of enamel lesion and concluded that theobromine-forming medium can enhance the remineralization potential of the tooth.[8] The amount of theobromine in a 1-ounce dark chocolate bar has a better effect on tooth hardness than a 1.1% prescription sodium fluoride treatment. However, the fluoride toothpaste has so many benefits such as increasing enamel strength and resilience; there is drawback of highly toxic if ingested or absorbed. There has never been a viable alternative to fluoride up until now theobromine is nonfluoride which is an alternative for fluoride toothpaste. The advantage of this theobromine contains nontoxic because of nonfluoride. A delicate blend of theobromine calcium and phosphate was added to maximize the enamel remineralization.

Sadeghpour and Carey found that theobromine protected teeth from decay better than fluoride. While fluoride is an effective enamel strengthened that can have some adverse effects such as dental fluorosis, or tooth discoloration, irritation of gastrointestinal at high doses. Conversely, theobromine was found to be readily absorbed by the gut, metabolized, and cleared cleanly by humans.[9]

Raloff stated that the theobromine is more active in hydrated silica, glycerin, and xylitol. "Xylitol is a sweetener that has garnered a lot of attention in oral care. Sodium bicarbonate, known to neutralize bacterial acids in the mouth, and titanium dioxide as a whitening agent and slight abrasive to help clean the surface of the tooth."[9] The titanium dioxide removes the bacteria and proteins that stick to the tooth and prepares the tooth to work more effectively. The two formulations are the same, save for the higher dose of active in one, which addresses compromised or weak enamel and extreme tooth sensitivity.[10]

Venkatesh Babu *et al*. reported that cocoa bean husk extract mouthrinse can be used as an alternate to chlorhexidine mouthrinse as they both have same antimicrobial effect and evades the side effects of later.[11]

Cocoa bean husk extract rinse is highly effective in reducing mutans streptococci counts and plaque accumulation when used as mouth rinse by children. Although no side effects were observed, the common complaint expressed by the children was bitter taste of the rinse. Addition of noncariogenic sugar substitutes should make it more acceptable, especially for children. Hence, the theobromine can be incorporated in chocolates, chewing gum, beverages, mouthwash, and toothpaste to prevent dental caries.[12]

In the present study, theobromine was used as it had been proven to be effective than fluoridated kids toothpastes. There was a greatest zone of inhibition in *S. mutans* counts, *L. acidophilus*, *and E. faecalis*, but unfortunately, it was least with *C. albicans*. These findings show that theobromine significantly reduced plaque deposition and prevents the caries as *C. albicans* does not play a major role in the carious process. Hence, this *C. albicans* is disadvantage of theobromine.

CONCLUSION

Theobromine though nonfluoridated toothpaste showed greater zones of inhibition which indicates that it is more effective than commercially available fluoride kids toothpastes.

Theobromine has got the capacity to reduce the plaque deposition and prevents the caries. Theobromine can be used as an alternative for fluoridated toothpastes which is safe to swallow and nontoxic for children.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

REFERENCES

1. Bhati N, Jaidka S, Somani R. Evaluation of antimicrobial efficacy of *Aloe vera* and meswak containing dentifrices with fluoridated dentifrice: An *in vivo* study. J Int Soc Prev Community Dent 2015;5:394-9.
2. George D, Bhat SS, Antony B. Comparative evaluation of the antimicrobial efficacy of *Aloe vera* tooth gel and two popular commercial toothpastes: An *in vitro* study. Gen Dent 2009;57:238-41.
3. Sudharsana, Srisakthi. Tooth friendly chocolate. J Pharm Sci Res 2015;7:49-50.
4. Srikanth RK, Shasikiran N, Subbha Reddy VV. Chocolate mouth rinse: Effect on plaque accumulation and mutans streptococci counts when used by children. J Indian Soc Pedod Prev Dent 2008;26:67-70.
5. Kamal Badiyani B, Kumar A, Bhat PK, Sarkar S. Chocolate disinfectant: Effectiveness of cocoa bean husk extract on *Streptococcus mutans* in used toothbrushes. Int J Oral Care Res 2013;1:7-10.
6. Osawa K, Miyazaki K, Shimura S, Okuda J, Matsumoto M, Ooshima T, *et al.* Identification of cariostatic substances in the cacao bean husk: Their anti-glucosyltransferase and antibacterial activities. J Dent Res 2001;80:2000-4.
7. Tarka SM Jr., Arnaud MJ, Dvorchik BH, Vesell ES. Theobromine kinetics and metabolic disposition. Clin Pharmacol Ther 1983;34:546-55.
8. Amaechi BT, Porteous N, Ramalingam K, Mensinkai PK, Ccahuana Vasquez RA, Sadeghpour A, *et al.* Remineralization of artificial enamel lesions by theobromine. Caries Res 2013;47:399-405.
9. Arman S. A neural network analysis of theobromine vs. fluoride on theenamel surface of human teeth. Diss Abstr Int 2007;68 Suppl B: 150.
10. Raloff J. Chocolate Constituent Bests Fluoride. Web Edition. Available from: http://www.sciencenews.org/view/generic/id/8545/description/Chocolate_Constituent_Bests_Fluoride. [Last accessed on 2007 May 22].
11. Venkatesh Babu NS, Vivek DK, Ambika G. Comparative evaluation of chlorhexidine mouthrinse versus cacao bean husk extract mouthrinse as antimicrobial agents in children. Eur Arch Paediatr Dent 2011;12:245-9.
12. Anderson K. Theobromine for tooth decay prevention. eCosmetics & Toiletries Magazins. Schaefer; 2012.