Original Article

Effect of medicated toothpastes and Tooth Mousse on cariogenic microbes of the oral cavity: An *in vitro* study

Mithra N. Hegde, Nishanth N. Hegde, Preethesh Shetty, Nishmitha N. Hegde, Chaithra Lakshmi Vittal, Manikandan Ravinanthan[1]

Department of Conservative Dentistry and Endodontics, AB Shetty Memorial Institute of Dental Sciences, Nitte (Deemed to be University), Mangalore, Karnataka, India, [1]Department of Restorative Dental Sciences, Faculty of Dentistry, Al Baha University, Al Baha, Saudi Arabia

Abstract

Background: Dental caries, commonly known as tooth decay is a widespread oral health problem mainly attributed to the activity of cariogenic bacteria, such as *Streptococcus mutans* and *Lactobacillus* species. Tooth Mousse, containing remineralizing agents, herbal and fluoride containing toothpaste with antimicrobial agents have been developed to target cariogenic bacteria. Herbal, fluoride toothpaste, and Tooth Mousse are commonly prescribed to prevent, reduce, and control dental caries.

Aim: This study aims to analyze the effect of Tooth Mousse and medicated toothpastes on *S. mutans* and *Lactobacillus acidophilus* using direct contact test.

Methodology: *L. acidophilus* and *S. mutans* were cultured on Mueller–Hinton agar (MHA-Hi media) using sterile cotton swabs and plates were dried for 15 min. Toothpastes (Dabur Red, Pepsodent) and Tooth Mousse were used at 1:1 dilution using sterile pyrogen-free distilled water. Fifty microliter of toothpastes and Tooth Mousse were introduced into each well. The plates were incubated at 37°C for 24 h.

Results and Discussion: The antimicrobial activity was evaluated by measuring the diameter of zones of inhibition (mm). The toothpaste containing fluoride (A) showed greater zone of inhibition compared to herbal toothpaste (B) whereas Tooth Mousse (C) did not show any zone of inhibition.

Conclusion: Among herbal and fluoride toothpaste, fluoride containing toothpaste showed more zone of inhibition thereby attributing to its increased antimicrobial property on S. mutans and L. acidophilus.

Keywords: *Lactobacillus acidophilus*; oral cavity; *Streptococcus mutans*; toothpastes

INTRODUCTION

Dental caries, commonly known as tooth decay is a widespread oral health problem mainly attributed to the activity of cariogenic bacteria, such as *Streptococcus mutans* and *Lactobacillus* species. Oral hygiene measures are effective in preventing caries.

The process of caries is dynamic and complex, with many different variables contributing to and starting the disease's progression. It is brought on by a specific class of acid-producing bacteria, specifically *Lactobacillus* and *S. mutans* group, which wreak havoc when fermentable carbohydrates

Address for correspondence:
Dr. Mithra N. Hegde,
AB Shetty Memorial Institute of Dental Sciences, Nitte (Deemed to be University), Mangalore, Karnataka, India.
E-mail: drhegdedentist@gmail.com

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such as sucrose, fructose, and glucose are present. Therefore, the main microbes implicated in tooth caries and their levels in the oral cavity should be decreased to prevent dental caries.[1]

Fluoride containing toothpaste (Pepsodent) has abrasives such as silica, calcium carbonate ($CaCO_3$) and binders like sodium carboxymethylcellulose. It also contains foaming ingredients like sodium lauryl sulfate which helps to lower the surface tension and thereby the toothpaste can contact the teeth easily.[2]

Herbal toothpaste (Dabur Red Paste) contains herbal ingredients such as clove oil, its main component, eugenol (70%–90%), gives it antibacterial qualities that help prevent toothache. It is known as Mukha Shodhak (Cleans the mouth) in Ayurveda and is effective in treating gingivitis.[3]

Tooth Mousse, containing remineralizing agents balances the oral pH and prevents caries. These ingredients are believed to have anticariogenic effects, meaning they help prevent tooth decay. Tooth Mousse exhibits unique properties of remineralization, buffering effect, casein phosphopeptide (CPP), reduction of plaque acidity, and enhanced fluoride uptake.

Tooth Mousse helps to remineralize tooth enamel. CPPs are bioactive agents that localize ACPF by preserving a reservoir of calcium, phosphate, and fluoride ions precisely where it is needed. This allows CPP-ACPF nanocomplexes to be formed, which reduce enamel demineralization (DEM) and enhance remineralization (REM).[4]

Calcium and phosphate ions in the Tooth Mousse can replenish lost minerals in the enamel, making it stronger and more resistant to acid attacks. This remineralization process can help repair early stages of tooth decay, known as demineralization.[5] Tooth Mousse can reduce plaque acidity. Acidic plaque contributes to the demineralization of enamel and the development of cavities. By neutralizing acids and providing minerals, Tooth Mousse helps maintain a healthier oral environment.[6] When applied topically by dental professionals or by the patient after tooth bleaching, CPP-ACP, a remineralizing agent developed by the American Dental Association Foundation to remineralize teeth and reverse early enamel carious lesion, has been shown to make teeth less sensitive to hot, cold, air pressure, and tactile stimulation.[7]

Overall, Tooth Mousse can be effective in preventing tooth decay and promoting oral health when used as part of a comprehensive dental care routine.

Caries is a disease of the hard tissues of the teeth caused by an imbalance, overtime, in the interactions between cariogenic bacteria in dental plaque and fermentable carbohydrates. Regular toothbrushing or external application of remineralizing agents is the principal nonprofessional intervention to prevent caries, but the caries preventive effect varies according to varied composition.

Kooshki *et al*. studied the antibacterial activity of Iranian herbal toothpaste and compared its chemical counterpart at three concentrations of 1, 1:1, and 1:3, respectively, on *S. mutans*, *Lactobacillus*, and *Candida albicans*. As the concentration of the herbal toothpaste decreased, the antibacterial efficacy dramatically decreased in comparison to the chemical toothpaste.[8] Kurian and Geetha found that fluoridated toothpaste has the highest level of antibacterial activity at all concentrations. The presence of various ingredients such as triclosan and fluoride in fluoride-containing toothpaste may be the cause of its increased antibacterial action.[9] The present study aimed to evaluate the antimicrobial activity of herbal toothpaste, fluoride containing toothpaste and Tooth Mousse on *Lactobacillus acidophilus*, and *S. mutans* of the oral cavity in an *in vitro* setting.

METHODOLOGY

Study Design and Setting

The present *in vitro* study was conducted at microbiology laboratory.

Bacterial strains of *S. mutans* (ATCC 35668) and *L. acidophilus* (ATCC 4356) were obtained in lyophilized from HiMedia Laboratories Pvt. Ltd. The antimicrobial activity of fluoride toothpaste (A), herbal toothpaste (B), and a GC Tooth Mousse (C) were compared [Table 1]. Muller–Hinton agar (MHA HiMedia) was prepared by dissolving 5.7 g of MHA in 150 mL of distilled water autoclaved at 121°C for 15 min and cooled to 45°C–50°C, mixed well and poured into sterile

Table 1: Classification of toothpaste and their components

Group	Toothpaste	Company	Ingredients
A	Pepsodent	Unilever	$CaCO_3$, sorbitol, hydrated silica, sodium lauryl sulfate, sodium monofluorophosphate, cellulose gum, benzyl alcohol, potassium nitrate, sodium saccharin, sodium silicate, cetylpyridinium chloride, limonene
B	Dabur Red Paste	Dabur India Limited	Contains various natural and herbal ingredients derived from ginger, clove, pepper, Meswak, mint, cinnamon, cardamom, amla, neem, tulsi, babool, *Aloe vera*, etc.
C	GC Tooth Mousse	GC, Tokyo, Japan	Pure water, glycerol, CPP-ACP, D-sorbitol, CMC-Na, propylene glycol, silicon dioxide, titanium dioxide, xylitol, phosphoric acid, flavoring, zinc oxide, guar gum, propyl p-hydroxybenzoate, butyl p - hydroxybenzoate

CPP: Casein phosphopeptide, ACP: Amorphous calcium phosphate, CMC-Na: Sodium carboxymethylcellulose

petri plates. *L. acidophilus*, *S. mutans* were cultured on MHA HiMedia using sterile cotton swabs and plates were dried for 15 min. The fluoride toothpaste, herbal toothpaste and Tooth Mousse were prepared at 1:1 dilution using sterile pyrogen-free distilled water. Three wells were made on all six plates using sterile micro tips and 50 µL of toothpastes and Tooth Mousse were introduced into each well (Pepsodent-Hindustan Unilever Ltd., Haridwar, Uttarakhand, India; Dabur Red Paste- Dabur India Ltd, Udham Singh Nagar, Uttarakhand, India; GC Tooth Mousse- GC, Tokyo, Japan). The plates were incubated at 37°C for 24 h. The antimicrobial activity was evaluated by measuring the diameter of zones of inhibition (mm) using digital caliper.[9]

Statistical analysis

Data were analyzed using Independent *t*-test. Statistical software SPSS version 20 was used to analyze the data. A $P < 0.05$ was considered statistically significant.

RESULTS

The antibacterial activity of two toothpastes and a Tooth Mousse at 50 µL was screened by agar well diffusion technique and the zone of inhibition was measured in mm diameter using digital caliper. The GC Tooth Mousse (C) did not show any zone of inhibition (0 mm) for both *S. mutans* and *L. acidophilus* Figures 1 and 2.

The results of group A and B are given in Tables 2 and 3. Fluoride toothpaste (A), was more effective against *S. mutans* and *L. acidophilus* with an average zone of inhibition of 40 mm and 30 mm diameter, respectively. Herbal toothpaste (B) showed an average zone of inhibition of 22 mm diameter for *S. mutans* and 21 mm diameter for *L. acidophilus*. The mean zone of inhibition is higher in group A compared to group B and it is statistically significant ($P < 0.001$) for *S. mutans* and $P = 0.005$ (<0.05) for *L. acidophilus*.

DISCUSSION

Dental caries is thought to be mostly caused by *S. mutans*, especially in regard to disease onset. These pioneering streptococci produce compounds and signals which attract in and facilitate the colonization of subsequent bacterial species.[10]

For more than a century, lactobacilli have been implicated as significant causes of dental caries in humans.[11] Due to their adeptness at fermenting sugar, *S. mutans* and *Lactobacillus* spp. are regarded as important etiological organisms in the cariogenic supragingival biofilm. Saliva samples from patients suffering from dental caries and periodontitis showed a higher relative abundance of these particular and well-known disease-associated bacterial species.[12]

Table 2: Effect of Group A and B on *Streptococcus mutans*

Groups	Mean	SD	*t*	*P*	95% CI of the difference	
					Lower	Upper
Zone of inhibition (mm)						
A	40.00	1.73	15.588	<0.001***	14.79	21.21
B	20.00	1.00				

***$P<0.05$ statistically significant. CI: Confidence interval, SD: Standard deviation

Table 3: Effect of Group A and B on *Lactobacillus acidophilus*

Groups	Mean	SD	*t*	*P*	95% CI of the difference	
					Lower	Upper
Zone of inhibition (mm)						
A	30.00	2.00	5.511	0.005**	4.47	13.53
B	21.00	2.00				

**$P<0.05$ statistically significant. CI: Confidence interval, SD: Standard deviation

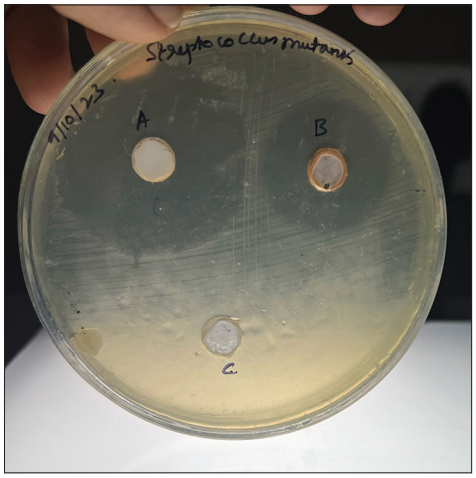

Figure 1: Antimicrobial activity of toothpastes and Tooth Mousse against *Streptococcus mutans*

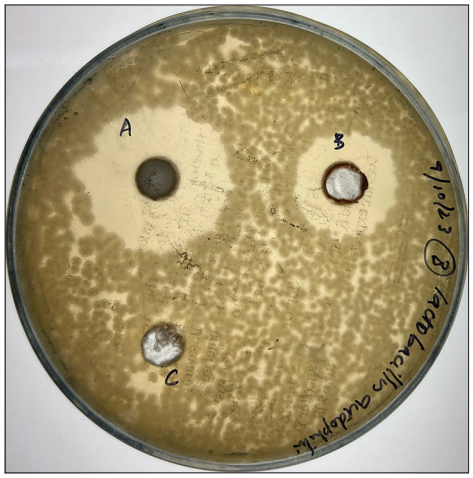

Figure 2: Antimicrobial activity of toothpastes and Tooth Mousse against *Lactobacillus acidophilus*

The aim of the study was to compare the antimicrobial activity of fluoride containing toothpastes, herbal toothpaste and a Tooth Mousse against *S. mutans* and *L. acidophilus*.

The fluoride toothpaste (A) contains $CaCO_3$, and silica which acts as an abrasive and helps in removing materials attached to the surface of the teeth. The foaming ingredient, sodium lauryl sulfate helps in spreading of toothpaste throughout the oral cavity to maximize cleansing power and to remove internal debris by functioning as a surfactant. These surfactants lower the surface tension of the liquid environment in the oral cavity so that the substances in the toothpaste can contact the teeth more easily.[13] It has been discovered that fluoride in toothpaste inhibits the growth of bacteria, especially *S. mutans*, which is a primary cause of dental caries. The presence of ingredients such as triclosan and fluoride, which have been demonstrated to have maximum antimicrobial action at different doses, is what gives fluoride toothpaste its antimicrobial efficacy.[14-16] As per the results shown in Tables 2 and 3, group A-fluoride containing toothpaste showed the highest antibacterial property with the greatest zone of inhibition.

Herbal toothpaste (B) contains natural and herbal ingredients derived from ginger, clove, pudina satva and karpura (camphor): Pudina's major ingredient, menthol, has anti-inflammatory and anti-germ qualities that contribute to its ability to keep breath fresh. Due to its slight local anesthetic effect,[17] Karpura (Camphor) reduces the pain and sensitivity associated with dental caries. Because piperine has anti-fungal qualities, pippali with piperine helps to maintain oral hygiene.[18] Herbal ingredients reduce enamel demineralization and inhibits the growth of bacteria contributing to improved oral health.[19] In a study by Patil *et al*., in 2022 showed that aloe vera extract helps in the reduction of colony forming units of *Enterococcus faecalis*.[20]

The antibacterial activity seen in group B was greater than group C, since group C did not show any zone of inhibition.

Tooth Mousse (C) can buffer acidic conditions in the mouth. When we consume acidic foods and beverages, the pH level in the mouth decreases, creating an environment conducive to tooth decay. Calcium and phosphate ions in Tooth Mousse can neutralize acids, reducing their harmful effects on tooth enamel. CPP in Tooth Mousse helps stabilize calcium and phosphate ions in a soluble form. This enhances their ability to penetrate the tooth enamel, providing better remineralization. Tooth Mousse was effective in clinical studies in decreasing caries. However, direct contact test suggested that antibacterial activity of Tooth Mousse is negligible. This could be because of the pH of Tooth Mouse which is 7 and above helping in the buffering in the oral cavity and reduction in caries.[6,21] In the present study with direct contact test, it did not show any results.

In the present study, we observed that fluoride containing toothpaste was more effective than herbal toothpaste as it showed more zone of inhibition for *S. mutans* and *L. acidophilus*. Fluoride has antibacterial activity which helps in the decrease of *S. mutans* and *L. acidophilus*.[22] These findings are similar to the study conducted by Kurian and Geetha[9] and Maripandi *et al*.[23] Tooth Mousse did not show any zone of inhibition against S. mutans and L. acidophilus, which is similar to the study conducted by Gokhale (2017).[24]

Studies suggest that CPP-ACP may enhance the uptake of fluoride, another mineral important for remineralizing enamel and preventing cavities. The combination of fluoride and CPP-ACP can provide synergistic benefits for dental health.[25]

Limitation

The antimicrobial effect of toothpastes and Tooth Mousse in different dilutions, and in different concentrations was not analyzed.

The intra oral environment in the presence of artificial saliva needs to be studied.

CONCLUSION

To clean, maintain, and improve the appearance and health of teeth, toothpaste is a paste or gel dentifrice used in conjunction with a toothbrush. There are numerous chemicals found in plants used in traditional medicine that can be utilized to treat both infectious and chronic ailments. Among herbal and fluoride toothpaste, fluoride containing toothpaste showed more zone of inhibition thereby attributing to its increased antimicrobial property on *S. mutans* and *L. acidophilus*. Tooth Mousse had no effect on *S. mutans* and *L. acidophilus* in the direct contact test method of evaluating antimicrobial activity.

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Conflicts of interest

There are no conflicts of interest.

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