M 08

Effects of Chlorhexidine and Clove Extract Oral Rinses on Dental Plaque Microbes

Himratul-Aznita W.H. and Zainal Abidin Z.
Department of Oral Biology, Faculty of Dentistry, University of Malaya, 50603 Kuala Lumpur.
*Corresponding author’s email: aznita@um.edu.my

Dental plaque is a thin film of microorganisms on the tooth surfaces and contributes to the development of dental caries and periodontal disease. It has been widely known that the usage of antimicrobial oral rinses plays an important role in maintaining oral hygiene mainly by reducing the numbers of dental plaque microbes. This study has been carried out with the aim of comparing the clinical effectiveness of chlorhexidine (CHX) (Oradex®) and commercialized clove extract (Mustika Ratu®) oral rinses in reducing dental plaque microorganisms. In addition, the aim was also to determine the duration of effects and magnitude of suppression of plaque microbes with the usage of the oral rinses. The antibacterial effects of CHX and clove extract oral rinses of dental plaque microbes were evaluated in healthy adults, which were not on any antibiotic treatment for the past 6 months. The oral rinses were tested on volunteers for a period of 30 minutes interval for up to 120 minutes. Volunteers were required to suspend normal oral hygiene habits and on the the sampling day, the tooth surfaces of each volunteer were swabbed, followed by rinsing with the specified oral rinse. The tooth surfaces were swabbed again after 30, 60 and 120 minutes. Swab samples were serially diluted and plated on BHI agar. Phenotypic appearance and the colony forming units (CFU/ml) were obtained. Results indicated that bacterial colony formation was significantly inhibited by both oral rinses and exhibited similar levels of antibacterial activity. However, the oral rinse containing CHX exhibited a longer duration of activity in controlling oral microbes compared to clove extract oral rinse. In conclusion, CHX has a better antimicrobial action to reduce and exhibit longer magnitude of suppression of dental plaque microbes than clove extract oral rinse.

M 09

Anti-parasitic effects of nerolidol on the morphology of Trypanosoma evansi in mice.

Mohd Shukri Hj Baba 1 & Zainal-Abidin B.A.H. 2
School of Biosciences & Biotechnology, Faculty of Science & Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia
*Corresponding author: Zainalab@phrsc.ukm.my

Morphological changes in cells are normally considered as the indirect evidence of the effect of test materials on targeted cells. In this study, the anti-parasitic activities of nerolidol (3,7,11-trimethyl-1, 6, 10-dodecatrienol), an active compound of the herb Elletaria cardamomum, was compared with the effect of benenil (commercially available anti-trypanosomal drug) on the morphology of a protozoan parasite Trypanosoma evansi in mice. Groups of male ICR strain mice were subjected to infection with the parasite (5.0 × 10^5 T. evansi per mouse) or without infection or treated intraperitoneally with nerolidol (0.1ml 1:10 solution per mouse) or benenil (0.01ml per mouse) or distilled water (as control), concurrently with the infection. Blood samples were collected subsequently following the treatment and prepared both for the normal observation under light microscope or for observations using electron microscopy. All mice in the negative group (untreated infection) succumbed to
Effects of Chlorhexidine and Clove Extract Oral Rinses on Dental Plaque Microbes

Himmatul-Azrina W.H. and Zainal Abidin Z.

Department of Oral Biology, Faculty of Dentistry, University of Malaya, 50603 Kuala Lumpur.

*Corresponding author’s email: azrina@um.edu.my

Abstract

Dental plaque is a thin film of microorganisms on the tooth surfaces and contributes to the development of dental caries and periodontal disease. It has been widely known that the usage of antimicrobial oral rinses plays an important role in maintaining oral hygiene mainly by reducing the numbers of dental plaque microbes. This study has been carried out with the aim of comparing the clinical effectiveness of chlorhexidine (CHX) (Oradex®) and commercialized clove extract (Mustika Ratu®) oral rinses in reducing dental plaque microbial counts. In addition, the aim was also to determine the duration of effect and magnitude of suppression of plaque microbes with the usage of the oral rinses. The antibacterial effects of CHX and clove extract oral rinses of dental plaque microbes were evaluated in healthy adults, which were not on any antibiotic treatment for the past 6 months. The oral rinses were tested on volunteers for a period of 30 minutes interval for up to 120 minutes. Volunteers were required to suspend normal oral hygiene habits and on the the sampling day, the tooth surfaces of each volunteer were swabbed, followed by rinsing with the specified oral rinse. The tooth surfaces were swabbed again after 30, 60 and 120 minutes. Swab samples were serially diluted and plated on BHI agar. Phenotypic appearance and the colony forming units (CFU/mL) were obtained. Results indicated that bacterial colony formation was significantly inhibited by both oral rinses and exhibited similar levels of antibacterial activity. However, the oral rinse containing CHX exhibited a longer duration of activity in controlling oral microbes compared to clove extract oral rinse. In conclusion, CHX has a better antimicrobial action to reduce and exhibit longer magnitude of suppression of dental plaque microbes than clove extract oral rinse.

Introduction

The attachment of a thin film containing microorganisms on tooth surfaces are known as dental plaque or dental biofilm (Socransky & Haffajee, 2002), that contributes to the development of oral diseases such as dental caries and periodontal disease. In the mouth, bacteria can attach to a wide variety of surfaces, including the soft tissues, the pellicle-coated teeth and other bacteria. Dental plaque microbes exhibit unique properties conferred by their site of colonization and the nature of the environment in which they reside.

The importance of effective dental plaque control has been emphasized over the years. As an adjunct to mechanical methods for dental plaque elimination, oral rinse products containing chemotherapeutic agents with a variety of antimicrobial mechanism have been beneficial and desirable (Azellson & Lindhe, 1978). There are two types of oral rinse; that is medicated and non medicated oral rinses. Medicated oral rinse usually contains chlorhexidine gluconate, having pronounced as antiseptic properties, which could inhibit the formation of dental plaque (Elworthy et al, 1996). Chlorhexidine has a wide-spectrum antibacterial activity encompassing Gram positive and Gram negative
bacteria, yeasts and other microbes. The non-medicated oral rinse usually contain herbal extracts such as Phenol, Thymol, Eugenol (derived from the buds of cloves), and many others (Elworthy et al., 1996). Such important compounds can be isolated from plant sources and are safe for humans.

Oral rinse products are capable of reducing the rate of dental plaque formation to a statistically significant degree. However, the information on the inhibitory duration effect on the plaque insufficient (Christine et al., 2000). Therefore, this study has been carried out with the objective of comparing the clinical effectiveness of chlorhexidine (CHX) (Oradex) and commercialized clove extract (Mustika Ratu) oral rinses in reducing dental plaque microorganisms. In addition, the aim was also to determine the duration of effects and magnitude of suppression of dental plaque microbes with the usage of the oral rinses.

Materials & Methods

Oral Rinses
Commercialized oral rinses used in the experiment are Oradex that contains 0.12% chlorhexidine gluconate as the active compound, and Mustika Ratu that contains clove extract as its active compound. Distilled water was used in the experiment as a negative control.

Rinsing Procedures
The antibacterial effects of chlorhexidine and clove extract on dental plaque microbes were evaluated on dentally healthy adults which were not on any antibiotic treatment for the past 6 months. The used rinsing volume of each of the oral rinse was based on the suggested instructions on the manufacturer's product labels. Volunteers were required to suspend normal oral hygiene habits daily, which include the routine toothbrushing every morning after getting up from bed and before bedtime.

Sampling Procedures
On the sampling day, the tooth surfaces of each volunteer were swabbed with sterile cotton swabs followed by rinsing with the specified oral rinse. The tooth surfaces were swabbed again after 30, 60 and 120 minutes. Swab samples were vortexed for 30 seconds to dislodge all microbes. Following this, samples were serially diluted and plated on Brain Heart Infusion (BHI) agar. All plates were incubated at 37°C for 18-24 hours.

Bacterial Identification
Pure colonies were isolated and Gram stained for cell morphology identification. The isolates were then subjected to bacterial identification procedures using the API Identification System (BioMerieux, France).

Results and Discussion

Three different types of bacterial colonies were successfully cultured on the BHI agar plates. Colonies were yellowish, white and cream in colour. The yellowish colonies had diameter approximately ranging between 1mm-2mm which were identified as Staphylococcus species, whereas the cream colour colonies with diameter ranging from 1-2mm were identified as Streptococcus species, and the white colonies which were around 4-5mm in diameter were identified as Candida species.
Results from the study also indicated that rinsing with the oral rinse showed reduction in the dental plaque population (Table 1). 0.12% chlorhexidine showed a significant reduction in the bacterial population of the dental plaque microbes. For the first 30 minutes after rinsing, 79.8% of the dental plaque microbes were reduced by chlorhexidine. Rinsing with clove extract showed a weaker effect compared to chlorhexidine, when about 72.3% of the dental plaque microbes were killed. In contrast, rinsing with distilled water did not produce a significant falls to the plaque microbes and had given the least reduction when only 21.5% were reduced.

Chlorhexidine has shown to exhibit longer suppression effect towards dental plaque microbes up to the tested duration period of 120 minutes (Graph 1). In contrast, the clove extract exhibited shorter duration effect, and was only able to control the suppressive effect for 60 minutes, after which the plaque microbes had started to increase and less reduction percentage observed. Distilled water however, was not able to reduce the plaque microbes and thus there were no changes observed during the 120 minutes.

<table>
<thead>
<tr>
<th>Time</th>
<th>Oral rinse (CFU/ml)</th>
<th>% reduction</th>
<th>Clove extract (CFU/ml)</th>
<th>% reduction</th>
<th>Distilled water (CFU/ml)</th>
<th>% reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>$1.83 \times 10^4$</td>
<td>$-%$</td>
<td>$1.73 \times 10^3$</td>
<td>$-%$</td>
<td>$1.58 \times 10^5$</td>
<td>$-%$</td>
</tr>
<tr>
<td>30 min</td>
<td>$0.37 \times 10^4$</td>
<td>$79.8%$</td>
<td>$0.48 \times 10^3$</td>
<td>$72.3%$</td>
<td>$1.24 \times 10^5$</td>
<td>$21.52%$</td>
</tr>
<tr>
<td>60 min</td>
<td>$0.38 \times 10^4$</td>
<td>$79.2%$</td>
<td>$0.59 \times 10^3$</td>
<td>$65.9%$</td>
<td>$1.42 \times 10^5$</td>
<td>$10.13%$</td>
</tr>
<tr>
<td>120 min</td>
<td>$0.40 \times 10^4$</td>
<td>$78.1%$</td>
<td>$1.34 \times 10^3$</td>
<td>$22.5%$</td>
<td>$1.33 \times 10^5$</td>
<td>$15.82%$</td>
</tr>
</tbody>
</table>

Graph 1: CFU of dental plaque microbes following single rinse with chlorhexidine, clove extract and distilled water.
Dental plaque is considered the major aetiological factors in the development of gingivitis and periodontitis (Kormman, 1986). This study has shown that dental plaque community was composed of a variety of different microorganisms which is in agreement to other reported results elsewhere (Socransky & Haffajee, 2002). Daily usage of oral rinse is important to maintain the oral hygiene and prevent dental plaque accumulation. The effective control of dental plaque is very important so as to prevent disease progression such as gingivitis and periodontitis.

Oral rinse containing antimicrobial properties play an important role in maintaining oral health by killing cariogenic organisms and preventing plaque, gingivitis, and bad breath. In order to reduce and control dental plaque microbes, antimicrobial agents have been employed to help to prevent the development of these diseases (Wade & Addy, 1989) and are considered effective and safe. In the study, the two antimicrobial agents had been proven to reduce the dental plaque microbes significantly compared to distilled water. However, comparison between the two agents have revealed that chlorhexidine was significantly more effective in reducing the plaque microbes than herbal extract (clove extract). A number of common dental plaque microbes such as staphylococci and streptococci including Candida species have been reported to be highly susceptible to chlorhexidine (Addy, 1986). It is because this active antimicrobial agent could destabilize and penetrate bacterial cell membranes and could interfere with membrane function by inhibiting oxygen utilization that leads to cell death.

This study has also demonstrated that chlorhexidine exhibits a longer suppression effect compared to the clove extract active compound. As for eugenol, which is the antimicrobial compound for clove extract also possesses activity against both Gram positive and Gram negative bacteria, but it is less effective and has shorter duration period in controlling the dental plaque microbes. This is in agreement with other reported results that chlorhexidine performed almost equal killing effect to other antimicrobial agents in most oral rinses for up to 30 minutes after rinsing, but continued its microbicidal effect for a longer duration period.

Conclusion

The result of this study has demonstrated that dental plaque microbes can be successfully suppressed by the use of oral rinse. Chlorhexidine and clove extract oral rinse can significantly reduce the microbial load of the dental plaque, with chlorhexidine provides higher antimicrobial activity in reducing and controlling the dental plaque microbes compared to clove extracts.

References


