ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)

IJDSIR : Dental Publication Service

Available Online at: www.ijdsir.com

Volume – 4, Issue – 6, December - 2021, Page No. : 242 - 246

Comparative evaluation of substantivity of 0.2% Chlorhexidine Gluconate and 10%Momordica charantia (bitter gourd) mouth rinses using spectrophotometric analysis: An in vitro study

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Citation of this Article: Dr Tejaswini M A, Dr Sunil S, Dr Pallavi Nanaiah, Dr Archana Naik, "Comparative evaluation of substantivity of 0.2% Chlorhexidine Gluconate and 10%Momordica charantia (bitter gourd) mouth rinses using spectrophotometric analysis: An in vitro study", IJDSIR- December - 2021, Vol. – 4, Issue - 6, P. No. 242 – 246.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Introduction: Chlorhexidine (CHX) is the gold standard chemical plaque control agent with the best known substantivity till date. Its anti-gingivitis and anti-plaque effect is well proven too. However, some of the adverse effects limits its usage and have encouraged the search for an alternative agent. Momordica Charantia (Bitter gourd) is a herbal alternative with several properties like anti-microbial and anti-inflammatory which may enhance oral hygiene. However, the property of substantivity is not known for this agent**.**

Aim and Objective: The aim of this study is to evaluate the substantivity of 10% Momordica Charantia mouthwash as compared with 0.2% chlorhexidine (CHX) mouthwash.

Materials and Methods: 102 Human enamel teeth surfaces were treated with 0.2% chlorhexidine gluconate and 10% Momordica charantia solutions for one minute. Fragments were placed in 4ml of artificial saliva and samples were collected at intervals of 8 hours and 12 hours and analyzed by spectrophotometry in the visible ultra violet region. Substantivity was evaluated by the measurement of absorbance in the visible ultra violet region for release of CHX or Momordica Charantia desorption from the treated tooth fragments in the artificial saliva.

Statistical analysis: The mean values obtained were statistically analyzed by Mann Whitney U Test and inter group analysis was done using Wilcoxon Signed Rank Test.

Results: The results showed that the concentration of 10% Bittergourd showed significantly higher mean substantivity values [0.610 ± 0.076] as compared to 0.2% chlorhexidine at 8 hours as well as 12 hours. (P =0.001) 0.2% chlorhexidine and 10% Bittergourd showed significantly higher mean substantivity values at 8 hrs time interval [0.571 ± 0.092] as compared to 12 hrs. (P <0.001)

Conclusion: These in vitro results show that the substantivity of 10% Bittergourd mouthwash appears to be slightly higher than chlorhexidine mouth wash.

Keywords: Chlorhexidine, Momordica Charantia, Substantivity, Spectrophotometry

Introduction

Frequent and precise removal of microbial plaque is the mainstay of prevention and treatment of almost all types of chronic gingival and periodontal diseases. Use of effective and safe adjuncts to supplement mechanical plaque removal is indicated for optimal plaque control whenever required.[1] Chemotherapeutic agents are used commonly and Chlorhexidine (CHX) is the gold standard chemical plaque control agent with high substantivity of 10–12 h against which the efficacy of newer antiplaque agents is compared because of its superior antiplaque effect and anti-gingivitis effect.[3] One of the main advantages of CHX, besides being a powerful antimicrobial, is its ability to bind to a wide variety of substrates while maintaining its antibacterial activity. It is then slowly released, leading to persistence of effective concentrations; this property is known as substantivity.[4] However, some of the adverse effects, such as dental pigmentation, mucosal erosions and drying of tissues and altering the taste,[5] limits its usage and have encouraged the search of an alternative agent.

Substantivity is defined as the prolonged adherence of the antiseptic to the teeth surface and its slow release in effective doses that guarantee the persistence of its antimicrobial activity. This property, together with the antimicrobial activity, is essential for an antiseptic to be clinically effective.[6]

Momordica Charantia (Bitter gourd) is an herbal alternative with several actions such as anti-diabetic, anti-microbial, and anti-inflammatory and may enhance oral hygiene.[7] Nevertheless, in vitro studies have demonstrated important effects of Momordica Charantia on pathogenic micro-organisms of the oral cavity that are found in the dental biofilm of teeth and dental caries including Streptococcus mutans and Lactobacillus acidophilus earlier, in the form of a gel.[8] Property of substantivity affords a prolongation of effect and provides continuous protection until the next rinse cycle is performed. The substantivity of antimicrobial agents in the oral environment is an important parameter for prevention of microbial plaque growth.

The purpose of this study was to determine the in vitro substantivity of Momordica Charantia (Bitter gourd) mouth wash and as compared with chlorhexidine mouthwash at different intervals of time.

Materials & Methods

Several techniques are available for the assessment of the efficacy of mouthwashes. The majority of previous studies have clinically investigated the effect of mouthwashes on prevention or reduction of microbial plaque. In the recent years, for the assessment of substantivity of mouthwashes, their effects on oral microbial flora have been investigated using microbial culture or fluorescence techniques.[1]The spectrophotometric analysis is newer technique and only

a limited number of studies have used this technique. It is more affordable than the culture technique and is less time consuming as well as economical. Very efficient in measuring concentration of solution, absorption band can also use to determine the concentration of compounds in solution.

Human teeth were sectioned with a water-cooled diamond saw to obtain the crowns. [fig.1] The 102 fragments obtained were randomly divided to evaluate the substantivity in Group 1, by one-minute immersion in 0.2% chlorhexidine gluconate solution and 10% Momordica charantia (Bittergourd) mouthwash in Group 2. [fig.2] The samples were immersed in 4ml of artificial saliva and kept in glass tubes. After 8 and 12-hour interval, an aliquot was taken from the tubes and the same volume was immediately replaced and analyzed by ultraviolet in a spectrophotometer at 260 nm [fig.3]. Substantivity was evaluated by the measurement of absorbance in the visible ultra violet region.

Statistical Analysis

The mean values obtained were analyzed by comparison of mean substantivity values between 2 groups at 8 and 12 hours time interval using Mann Whitney U Test and Comparison of mean substantivity values between 8 and 12 hours' time in CHX 0.2% and Bitter Guard 10% group using Wilcoxon Signed Rank Test.

Results

Table no 1 showed that, at 8 hrs time interval 10% Bittergourd showed significantly higher mean substantivity values [0.610 ± 0.076] as compared to 0.2% chlorhexidine [0.571 ± 0.092] at P=0.04. At 12 hrs time interval also, 10% Bittergourd showed significantly higher mean substantivity values [0.455 ± 0.068] as compared to 0.2% chlorhexidine [0.392 ± 0.103] at P=0. 001.Whereas inter group examination results showed that, CHX 0.2% showed significantly higher mean substantivity values at 8 hrs time interval [0.571 ± 0.092] as compared to 12 hrs [0.392 ± 0.103] at P<0.001. Similarly, 10% Bittergourd showed significantly higher mean substantivity values at 8 hrs [0.610 ± 0.076] as compared to 12 hrs [0.455 ± 0.068] at P<0.001. [Table no.2]

Table 1:Comparison of mean substantivity values between 2 groups at 8 and 12 hours' time interval using Mann Whitney U Test

Time	Groups	N	Mean	SD	Mean Diff	P-Value
8 Hrs	CHX 0.2%	51	0.571	0.092	-0.039	0.04*
	MC 10%	51	0.610	0.076		
12 Hrs	CHX 0.2%	51	0.392	0.103	-0.063	0.001*
	MC 10%	51	0.455	0.068		

* - Statistically Significant

Table 2: Comparison of mean substantivity values b/w 8 and 12 hours' time in CHX 0.2% and Bitter Guard 10% group using Wilcoxon Signed Rank Test

Groups	Time	N	Mean	SD	Mean Diff	P-Value
CHX 0.2%	8 Hrs	51	0.571	0.092	0.179	<0.001*
	12 Hrs	51	0.392	0.103		
BG 10%	8 Hrs	51	0.610	0.076	0.155	<0.001*
	12 Hrs	51	0.455	0.068		

* - Statistically Significant

Fig.1: Sectioned human teeth

Fig. 2: Immersion of teeth in 0.2% CHX and 10% Momordica charantia(Bitter gourd) mouthwash.

Fig. 3: A spectrophotometric analysis

Discussion

Progresses in science and pharmacology facilitate the access of dentists to a larger number of drugs every day. Studies have demonstrated that, in situ, 0.2% CHX has a greater immediate antibacterial effect and substantivity than other antiseptics used in the oral cavity.9 The persistence of CHX on the oral surfaces and its ability to suppress salivary bacterial counts was demonstrated to last for more than 12 hours. Thus, CHX in a mouth rinse (0.2% solution) is administered at 12-h intervals and retains its ability to retard/prevent plaque formation.10 In this in-vitro study CHX substantivity was noticed higher till the 8th hour and gradually decreased at 12th hour. Similar results were seen in the study *Freitas CS de et al*, 2 the maximum substantivity of chlorhexidine was observed after 60 minutes, as compared to 6 hours which was in accordance with results of our study.

In the present scenario, Momordica Charantia (Bitter gourd) is an herbal alternative with several actions such as anti-diabetic, anti-microbial, and anti-inflammatory and may enhance oral hygiene.7 many invitro studies shows effect of Momordica charantia on pathogenic micro-organisms of the oral cavity that are found in the dental biofilm of teeth. However, unlike CHX, substantivity of Momordica charantia was not evaluated so far. Hence, the present study was required to determine its substantivity thereby promoting its frequency of usage for the maximum efficiency.

The results of our study revealed that both 10% Momordica charantia performed better, with higher substantivity, at 12hours as compared to 0.2% CHX and these results were statistically significant. The better performance of 10% solution can be attributed to the antiplaque and anti-inflammatory activity of Momordica charantia at this concentration.

However, there was significant difference in the performance of 10% Momordica charantia and 0.2% CHX, at 8 hours as compared to 12hours. The rationale behind such a good performance of Momordica charantia at 10% concentration needs further exploration through clinical trials.

Conclusion

Based on in vitro experimental conditions of this work and within the limits, it can be concluded from our study that 10% Momordica charantia possess better significant substantivity than 0.2% CHX and thus can be used twice a day clinically as an antiplaque and anti-inflammatory agent against pathogenic micro-organisms of the oral cavity that are found in the dental biofilm of teeth.

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