Effect of small cardamom extracts on Mutans streptococci in comparison to chlorhexidine gluconate and de-ionized water (In vitro study)

Ghada A. Ibrahim, B.D.S. [(1)]
Wesal A. Al – Obaidi, B.D.S., M. Sc. [(2)]

ABSTRACT

Background: Herbs are being widely explored to discover alternatives to synthetic antibacterial agents.Small Cardamom often referred to as queen of spices because of its very pleasant aroma and taste, have a history as old as human race. Most people use cardamom as a spice and are largely unaware of its numerous health benefits. The purpose of this study was to evaluate the effect of different concentrations of water and alcoholic cardamom extracts on sensitivities, growth, and adherence of Mutans streptococci in vitro.

Materials and Methods: In this study, saliva was collected from ten volunteers (College students 18-22 years). Agar well technique was used to study the sensitivities of Mutans streptococci to different concentrations of small cardamom extracts and other control agents, also the effects of small cardamom extracts on viable counts, adherence of Mutans streptococci were studied

Results: According to agar well diffusion methods, both cardamom extracts were effective in inhibition of Mutans streptococci, but still weaker than chlorhexidine gluconate 0.2%. Alcoholic extracts showed higher zone of inhibition compared to the same concentration of water with high significance differences ($P<0.01$). The effects of 10%, 15%, and 20% of both water and alcohol extracts of small cardamom were tested on the viability counts of Mutans streptococci *in vitro*. Highly significant reduction in the counts of bacteria was reported of both cardamom extracts and CHX in comparison to control without agents after 24 hr. Both cardamom extracts less effective than CHX. All the concentration of water and alcohol cardamom mouth washes tested was not effective in prevention the adherence of bacteria on teeth surface *in vitro*, while CHX was effective in prevention the adherence of bacteria.

Conclusion: Cardamom extracts were effective against Mutans streptococci, but still less than CHX.

Keyword: Mutans Streptococci, Small Cardamom, Chlorhexidine, De-ionized water. (J Bagh Coll Dentistry 2013; 25(4):160-163).

الخلاصة

المقدمة: يجري استكشاف الإعشاب على نطاق واسع كبدائل لاصناعية مضادة للبكتريا .الهيل الأخضر هو ملك التوابل لما لة من راحة عطرة جدا وطعم, وله تاريخ قديم قدم الجنس البشري. معظم الناس يستخدمون الهيل كتوابل وغير مدركين فوائدة الصحية . الغرض من هذه الدراسة دراسة تأثير تراكيز مختلفة من المستخلص المائي والكحولي للهيل على الحساسية, النمو, والالتصاق لبكتريا المكورات المسبحية مختبريا.

المواد والعمل: في هذه الدراسة , تم جمع اللعاب من عشرة طلاب تتراوح أعمارهم بين (18-**22**). شملت التجربة اختبار حساسية الميوتانز للتراكيز المختلفة لمستخلص الهيل الأخضر والمواد الضابطة الأخرى بطريقة الانتشار من الحفر في الوسط البكتيري, كذلك تم دراسة تأثير مستخلص الهيل على النمو الحيوي للميوتانز وعلى قابلية البكتريا للالتصاق على الأسنان.

النتائج: حسب طريقة الانتشار من الحفر في الوسط البكتيريا مختبريا , كان المستخلصان المائي والكحولي فعلان في تثبيط هذه البكتريا. لكن يبقى تاثيرهما اقل من 0.2% كلورهكسدين كلوكونيت .المستخلص الكحولي كان له تأثير أقوى من المستخلص المائي بنفس التراكيز بفروق إحصائية عالية. ($P<0.01$) . تم اختبار تأثير تراكيز (10% و15% و20%) للمستخلص المائي والكحولي على النمو الحيوي للميوتانز مختبريا, ووجد أن مستخلص الهيل المائي والكحولي و الكلورهكسدين له فروقات إحصائية عالية في تقليل النمو الحيوي للبكتريا مقارنة بالنمو الحيوي للبكتريا بدون إضافة إي عامل بعد مرور 24 ساعة. لكن يبقى المستخلص المائي والكحولي للهيل اقل فعالية من 0.2% كلورهكسدين كلوكونيت. وجد ان جميع التراكيز المستخدمة لمستخلص الهيل المائي و الكحولي مختبريا غير فعالة في منع التصاق البكتريا على الأسنان وان 0.2 % كلورهكسدين كلوكونيت فعال في منع الالتصاق البكتريا.

الاستنتاج: أن مستخلص الهيل كان فعالا ضد بكتريا الميوتانز ولكن اقل تأثيرا من كلورهكسدين كلوكونيت.

كلمات مفتاحيه: المكورات المسبحية الميوتانز, الهيل الأخضر, الكلورهكسدين كلوكنيت, الماء الغير ايوني

INTRODUCTION

Dental caries is one of the most common infectious diseases in oral human cavity [(1,2)]. The mouth contains a wide variety of oral bacteria, but only a few species of bacteria are believed to cause dental caries; Mutans streptococcus and Lactobacilli [(3-5)]. The elimination of cariogenic bacteria from the oral cavity using antibacterial agents is one of primary strategies for prevention of dental caries [(6)]. Medicated oral rinses usually contains antimicrobial agents, such as chlorhexidine gluconate which is very potent chemo-prophylactic agent, it has abroad spectrum action especially against Mutans streptococci group. But it has many side effect like staining of teeth, altering the test of the mouth and desquamation of oral mucosa [(7,8)].

Herbs are being widely explored to discover alternatives to synthetic antibacterial agents [(9)]. Small cardamom often referred to as queen of spices because of its very pleasant aroma and taste, has a history as old as human race [(10)]. Most people use cardamom as a spice and are largely unaware of its numerous health benefits. In addition to its wide use for culinary purpose, cardamom has folkloric repute as carminative, stomachic, diuretic, antibacterial, analgesic, antiviral, anti-inflammatory, antifungal and is considered useful in treatment of many diseases [(10,11)]. There are very little exclusive studies about small cardamom antibacterial effect on dental caries. For all of the above this study was conducted.

(1) MSc student, Department of Pedodontics and Preventive dentistry, College of Dentistry, Baghdad University
(2) Professor, Department of Pedodontics and Preventive dentistry, College of Dentistry, Baghdad University

MATERIALS AND METHODS

Small cardamom fruits were obtained from AL-Shoorga market. Small cardamom fruits were American origin grade 4. The samples were carefully washed under de-ionized followed by sterile distilled water and then air dried for two days, pounded using a mixer grinder and stored in air tight bottles. There are two methods for extraction**:** water extraction and alcoholic extraction. For water extract 100grams of fruit powder of cardamom was soaked in 1000ml cold sterile distilled water in a conical flask and left undisturbed for 24h. For alcoholic extract 100 grams of fruit powder was kept in 70% ethanol for 3 consecutive days at room temperature. Then both extract filtered off using a sterile Whatman filter paper No1 [(12)].The filtered extract was concentrated under vacuum below 40^{o}C using a rotaevaporator. The weight of the solid residue was recorded and taken as the yield of crude extract [(13)]. Stimulated saliva was collected from ten healthy looking students from University of AL-Mustansiriya aged (18-22) years in order to carry out in vitro experiments from which Mutans streptococci were isolated, purified, and diagnosis according to morphological, microscopical, biochemical test and by VITEK2 test. Agar well technique was applied to study the antibacterial effects of different concentrations of water and alcoholic cardamom extracts (5%, 10%, 15%, 20%, 25%, 30%), compared with chlorhexidine 0.2% as a control positive and de-ionized water as control negative on MHA media.

These experiments were conducted on 10 isolates of Mutans streptococci**.** The viability counts of Mutans streptococci inoculated from broth media, to which 10%, 15%, and 20% of water and alcoholic cardamom extracts, CHX 0.2% and de-ionized water were added have been estimated in comparison to the control (broth and bacteria only).The procedure was carried on 5 isolates of Mutans streptococci. The prevention of adherence of Mutans streptococci to the teeth and stainless wire after the 10%, 15%, and 20% of water and alcoholic cardamom extracts, chlorhexidine 0.2% and de-ionized water compared to the control positive (broth and bacteria without agent) and control negative (broth and agent without bacteria) had been tested in vitro These experiments carried on 50 extracted first premolars (right and left sides) form Orthodontic department.

RESULTS

Sensitivities of Mutans streptococci (MS) to different concentrations of cardamom, CHX and de-ionized water in vitro were determined by using agar well diffusion method. The diameter of inhibition zone (clear zone of no growth of MS around each well) was found to increase as the concentrations of cardamom extracts increase. De-ionized water showed no zone of inhibition while CHX showed the highest zones of inhibition compared to the cardamom extracts as shown table (Table 1).Alcoholic extracts showed higher zone of inhibition compared to the same concentration of water with high significance differences ($P<0.01$) (Table 2).

The counts of MS were tested in vitro in the presence of 10%, 15%, and 20 % of water and alcoholic extracts of cardamom, CHX, de-ionized water and control. LSD test used to compare the initial count, the counts of bacteria after 24 hr and their counts after using different agents. The result showed high significance differences between agents except a significance difference between initial count and CHX and no significance difference between counts after 24and de-ionized water (Table 3). All the concentrations of cardamom extracts tested were failed in the prevention of adherence of Mutans streptococci, while control negative and teeth treated with CHX showed no accumulation of dental plaque on them after seen days of incubation (Table 4).

DISCUSSION

Sensitivities of Mutans streptococci to different concentrations of water and alcohol extracts of cardamom by agar well diffusion method had been tested in this study. Results showed that cardamom extracted by water and alcohol were able to inhibit the growth of Mutans streptococci, this finding were in coincidence with other studies [(10,14)].

The diameter of zones of inhibition of MS were increased as the concentration of both cardamom extracts increased from 10% to 30% but still lower than CHX 0.2%. For alcohol extract the zones of inhibition was much higher than water extract with highly significant differences, (this finding may be explained by the fact that, the components of cardamom that had antibacterial effects against MS and inhibit its growth, and were more soluble in alcohol than water. By laboratory analysis of small cardamom by HPLC (High-performance liquid chromatography), in this study, it was found that concentration of major active compounds (1-8 cineole, α-terpinyl acetate) is higher in alcohol extract than in water extract. The antimicrobial property of small cardamom has been shown to be attributable to the essential oil fraction [(15)].

A highly significant reduction was found in the viable counts of Mutans streptococci in 10%, 15% and 20% of both cardamom extracts compared to the control after 24 hr. It could be attributed to chemical constituents of small cardamom like Cineole; the major active component of cardamom oil. It is a potent antiseptic that is known to kill bacteria producing bad breath and other infections [10]. No one of any concentration of water and alcohol cardamom mouth washes tested was able to prevent adherence of bacteria. However in comparison to the control, plaque thickness was less. This reduction in plaque thickness may be attributed to the inhibitory effect of these agents on growth or metabolism of these bacteria rather than on adherence ability.

Table 1: Mean and SD of MS inhibition zone in millimeter to different concentration to different agents (Agar well diffusion methods)

Agents	No.	Mean	± S.D	ANOVA test
CHX	10	17.50	0.57	F=349.541 d.f=10 P=0.000 HS
Water extract 5%	10	0	0	
Water extract 10%	10	7.35	0.66	
Water extract 15 %	10	9.10	0.45	
Water extract 20 %	10	10.15	0.52	
Water extract 25 %	10	11.20	0.34	
Water extract 30 %	10	12.55	0.49	
Alcoholic extract 5 %	10	0	0	
Alcoholic extract 10 %	10	10.35	0.62	
Alcoholic extract 15%	10	12.20	0.63	
Alcoholic extract 20%	10	14.75	0.48	
Alcoholic extract 25%	10	16.20	0.78	
Alcoholic extract 30%	10	16.90	0.51	
De-ionized water	10	0	0	

Mean (mm).

Table 2: LSD test between sensitivity of Mutans streptococci to same concentration of both water and alcoholic extracts of cardamom (Agar well diffusion method)

Water extract	Alcoholic extract		
concentration	**Mean Difference**	**P-value**	**Description**
10%	-3.00	0.000	HS
15%	-3.10	0.000	HS
20%	-4.60	0.000	HS
25%	-5.00	0.000	HS
30%	-4.35	0.000	HS

Table 3: LSD between agents in comparison with initial counts and counts after 24

Agents	Initial Count			Count After 24		
	Mean Difference	**P-value**	**Description**	**Mean Difference**	**P-value**	**Description**
W.E 10%	172.6	0.000	HS	-82.0	0.000	HS
W.E. 15%	138.4	0.000	HS	-116.2	0.000	HS
W.E. 20%	94.2	0.000	HS	-160.4	0.000	HS
A.E. 10%	150.8	0.000	HS	-103.8	0.000	HS
A.E. 15%	104.0	0.000	HS	-150.6	0.000	HS
A.E. 20%	56.4	0.004	HS	-198.2	0.000	HS
D.W.	222.0	0.000	HS	-32.6	0.114	NS
CHX	-45.4	0.018	S	-300.0	0.000	HS

W.E= Water extract A.E= Alcoholic extract D.F=8

Table 4: The effects of cardamom, de-ionized water and CHX on adherence of MS in vitro

Agents (2 minutes)	Adherence
Control positive	+ve
Control negative	-ve
10 % water cardamom extract	+ve
15% water cardamom extract	+ve
20% water cardamom extract	+ve
10% alcoholic cardamom extract	+ve
15 % alcoholic cardamom extract	+ve
20 % alcoholic cardamom extract	+ve
CHX	-ve
De-ionized water	+ve

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