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

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ABSTRACT

Background: Small cardamom or green cardamom is the dried fruit of the tall perennial herbaceous plant, Elettaria cardamomum Maton belonging to the family Zingiberaceae. The major use of small cardamom on world wide is for domestic culinary purpose and in medicine. This study was conducted to test the effect of small cardamom extracts on Mutans streptococci and Candida Albicans in comparison to 0.2% chlorhexidine gluconate and de-ionized water *in vivo*.

Materials and Methods: Mutans streptococci and Candid Albicans were isolated, purified and diagnosed according to morphological characteristic and biochemical test. In this experiments, the effect of control agents and small cardamom extracts as a mouth rinses was tested on the saliva of group of volunteers to determine the level of Mutans streptococci and Candida Albicans in vivo. Also the salivary flow rate and pH were measured *in vivo*.

Result: 10 % aqueous cardamom extracts had a highly significant antimicrobial activity against mutans streptococci after 15 min after rinsing and following times. 30 % aqueous cardamom extracts had a significant antifungal activity in vivo against Candida albican after 30 min after rinsing and following times. But still CHX is more effective than the other agents in reduction the counts of mutans streptococci and Candida Albicans. CHX 0.2% mouth rinse had the highest stimulation of salivary flow rates and pH followed by hot water cardamom mouth rinse 30% followed by cold cardamom mouth rinse 10% then de-ionized water mouth rinse.

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

Keywords: Mutans Streptococci, Candida Albicans, Small Cardamom, Chlorhexidine, De-ionized water. (J Bagh Coll Dentistry 2013; 25(4):104-108).

الخلاصة

المقدمة: الهيل الصغير او الاخضر هو ثمرة مجففة لنبات عشبي معمر طويل القامة, الهال ماتون يعود إلى العائلة الزنجبيلية. إن الاستخدام الرئيسي للهيل على النطاق العالمي هو للإغراض الطهي المحلية وفي الطب. أجريت هذه الدراسة لاختبار تأثير مستخلص الهيل الأخضر على نمو بكتريا الميوتانز وفطر المبيضات بالمقارنة مع 0.2% كلوروهكسيدين كلوكونيت والماء الغير ايوني.

المواد والعمل: تم تنقية و تشخيص بكتريا المكورات المسبحية الميوتانز و فطر المبيضات حسب الصفات الشكلية و اختبارات الكيمياء الحياتية. في هذة التجربة تم اختبار تأثير المواد الضابطة و مستخلص الهيل الأخضر كغسول فم على مجموعة من المتبرعين لتحديد مستويات بكتريا المكورات العقدية الميوتانز وفطر المبيضات, كذلك تم قياس إفراز وقاعدية اللعاب سريريا.

النتائج.: مستخلص الهيل المائي بتركيز 10% سريريا له فرق إحصائي عالي في تقليل النمو الحيوي للبكتريا مقارنة بالماء الغير الايوني بعد 15 دقيقة من المضمضة به والأوقات التالية. مستخلص الهيل المائي بتركيز 30% سريريا له فرق إحصائي في تقليل النمو الحيوي لفطر المبيضات مقارنة بالماء الغير الايوني بعد 30 دقيقة من المضمضة به والأوقات التالية .0.2 .% كلورهكسدين كلوكونيت اكثر فعالية في تقليل النمو الحيوي لبكتريا المكورات المسبحية وفطر المبيضات.

الاستنتاج: ان مستخلص الهيل كان فعالا ضد بكتريا الميوتانز وفطر المبيضات ولكن اقل تأثيرا من0.2% كلورهكسدين كلوكونيت.

كلمات مفتاحيه: المكورات المسبحية الميوتانز, فطر المبيضات, الهيل الاخضر, الكلورهكسدين كلوكنيت, الماء الغير ايوني

INTRODUCTION

Dental caries is a dynamic process of demineralization of the dental hard tissues by products of bacterial metabolisms, alternating with periods of remineralization [(1)]. Mutans streptococci were found to be the predominant bacteria in caries process [(2-4)]. Different epidemiological and experimental studies showed a positive association between Mutans streptococci with initiation of carious lesion [(2,4-6)]. C. Albicans is the most common fungal pathogen in humans. C. Albicans can also act as an opportunistic pathogen with the ability to cause a variety of infections [(7)]. Some studies even have shown a significant association between C. Albicans and dental caries in children and young [(8,9)]. Colonization of the oral cavity by C**.** Albicans involves adherence of yeast cells to oral surfaces [(10)].

Treatment of dental caries and periodontal diseases need a lot of as cost as well manpower. Prevention, including use of chemical therapies, is more cost effective as patient shifts from high - risk to low-risk level [(11)]. Chlorhexidine is very potent chemo-prophylatic agent [(12)]. It has abroad spectrum action especially against Mutans streptococci group and Candida Albicans [(12,13)]. But it has many side effects [(12)]. Small cardamom or green cardamom, popularly known as 'Queen of Spices', is the dried fruit of the tall perennial herbaceous plant, Elettaria cardamomum Maton belonging to the family Zingiberaceae. The major use of small cardamom on world wide is for domestic culinary purpose and in medicine. The aroma and medicinal properties of cardamom are due to the volatile oil present in it [(14)].There are very little exclusive studies about small cardamom antibacterial effect on Mutans streptococci and Candida Albicans. For all of the above this study was conducted.

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MATERIALS AND METHODS

Small cardamom fruits were obtained from AL-Shoorga market. For Mutans streptococci we used cold water extract by disolve100 grams of fruit powder of cardamom in 1000ml cold sterile distilled water and left undisturbed for 24 then filtered [15].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 [16]. For Candida Albicans, we use hot water extract by dissolve 100grams of fruit powder of cardamom in 1000ml of sterile hot distilled water. The extract left for 48hr at room temperature then centrifuged at 2000 rpm for 10 min, then filtered. The extract was incubated at 37C until it became dry and stored in sterile screw capped vials in the refrigerator until needed [17]. Stimulated saliva was collected from ten healthy looking students from University of AL-Mustansiriya aged (18-22) from which Mutans streptococci and Candida Albicans were isolated, purified, and diagnosis according to morphological, microscopical, biochemical test and by VITEK2 test. The total number of volunteers were 24 and they were divided into 4 groups (each group was made up of 6 volunteers), the first group is the experimental group they used the water cardamom extract mouth rinse 10%, the second group is the experimental group they used the water cardamom extract mouth rinse 30 %, The third group used CHX 0.2% mouth rinse as control positive and the fourth group used de-ionized water mouth rinses as control negative.

Procedure:-

1.10 ml of 10%, 30% water cardamom extract, de-ionized water, and chlorhexidine gluconate 0.2% mouth wash were prepared.

2.Stimulated saliva was collected by chewing a piece of Arabic gum (0.5 gm) for 1 minute and then expectorate to remove all saliva then chewing a piece of gum (0.5 gm) for 1 minute and collecting the saliva in screw capped bottles[18].

Each participant was asked to rinse with aqueous solution for 1 minute, and then expectorate, stimulated salivary samples were recollected after 1 minute, 15minutes, 30 minutes, and 1 hour, during this time volunteers were asked not to eat or drink anything except water. Within less than 15 minutes, the pH of saliva was measured by the digital pH meter; also the volume of saliva was measured also. Sample of saliva were processed immediately, they were dispersed for 1 minute by vortex mixer, then 0.1 ml of saliva transferred to 0.9 ml of PBS, tenfold dilutions were performed. From the dilution 10^{-3}, 0.1 ml was taken and spread in duplicate on the surface of MSB and SDA agar plates, then incubated anaerobically for 48 hr at 37 ^{o}C, and aerobically for 24 hr at room temperature.

RESULTS

Mean counts of bacteria was estimated before and after rinsing with water extract 10%, CHX gluconate, de-ionized water. CHX had the maximum reduction in the bacterial viable counts followed by water cardamom extracts 10% while de-ionized water had the least reduction of bacterial counts among the agents as in Table (1). ANOVA test was used to examine the difference among the Mutans streptococci viable counts for the three mouth rinses at 5 time intervals. There no significant difference was found before rinsing while a significant difference after one minute of rinsing and a highly significant differences was found for the rest time point (Table1).Mean counts of C. Albicans was estimated before and after rinsing with water extract 30%, CHX gluconate, and de-ionized water. CHX had the maximum reduction in the Candida counts followed by water cardamom extracts 30% while de-ionized water had the least reduction of Candida counts among the agents as in Table (2). ANOVA test was applied to examine the difference among the Candida Albicans for the three mouth rinses at 5 time intervals. There were no significant differences before rinsing and after one minute and 15 minutes of rinsing while highly significant differences were found for the rest time point (Table 2). Salivary flow rate was increased immediately after rinsing for the four mouth rinses, rinsing with CHX and both cardamom extracts results in marked increased in the mean values of flow rates immediately after rinsing which continue for half an hour then started reduction (Table 3).

Salivary pH was increased immediately after rinsing for the four mouth rinses, rinsing with both cardamom extracts result in marked increased in the mean values of salivary pH immediately after rinsing which continue for half an hour then started reduction (Table 4). CHX 0.2% mouth rinse had the highest stimulation of salivary flow rates and pH followed by hot water cardamom mouth rinse 30% followed by cold cardamom mouth rinse 10% then de-ionized water mouth rinse. Alcoholic extract cause burning and discomfort of the mouth and the volunteers couldn't tolerate it. Therefore only water extract were used in vivo study.

DISCUSSION

Aqueous extract of cardamom 10% was tested for its effects on mutans streptococci colony forming unit counts among group of volunteers in comparison to de-ionized water and CHX. Cardamom extract had highly significant antimicrobial activity against Mutans streptococci as it can reduce the viable count of the bacteria profoundly in comparison to de-ionized water after 15 min after rinsing and following times. The reduction in the counts of the bacteria after 15 minutes after rinsing may be explained by the assumption that Mutans streptococci were sensitive to the antibacterial compounds present in cardamom extracts which continue to release in mouth after rinsing.

While still CHX is more effective than the other agents in reduction the mutans streptococci, this could be due to CHX having a prolonged bacteriostatic action and ability to adsorb into pellicle coated enamel surface and dental plaque during rinsing [19].Aqueous extract of cardamom 30% was tested for its effects on C. Albicans colony forming unit counts among group of volunteers in comparison to de-ionized water and CHX. Cardamom extracts had a significant antifungal activity against C. Albicans as it can reduce the viable count of the Candida profoundly in comparison to de-ionized water after 30 min after rinsing and following times. Many studies confirmed antifungal efficacy of small cardamom on C.albicans [20-22].

The antifungal activity of small cardamom on C. albicans is due to volatile oils whose main constituents are cineole, terpinol, and limonene [21]. But still CHX is significantly more effective than the other agents in reduction the counts of Candida Albicans. Salivary flow rates and pH increased immediately after rinsing for two cardamom mouth rinses which continue to increase for half an hour then gradually decreased to approximate the baseline after one hour. CHX 0.2% mouth rinse had the highest stimulation of salivary flow rates and pH followed by hot water cardamom mouth rinse 30% followed by cold cardamom mouth rinse 10% then de-ionized water mouth rinse.

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Table 1: Mean and standard deviation of MSX10^4 of three mouth washes in vivo.

Time	Agents	No.	Mean	± SD	F	P	Description
base	CHX	6	268.5	19.68	3.059	0.077	NS
	D.W.	6	268.0	24.65			
	W.E. 10%	6	234.0	36.13			
1 min	CHX	6	204.83	29.56	4.778	0.025	S
	D.W.	6	254.17	21.48			
	W.E. 10%	6	213.66	35.65			
15 min	CHX	6	154.83	26.69	18.902	0.000	HS
	D.W.	6	245.0	15.42			
	W.E. 10%	6	196.33	31.44			
30 min	CHX	6	116.33	22.09	49.363	0.000	HS
	D.W.	6	253.83	25.65			
	W.E. 10%	6	179.0	24.11			
1 hr	CHX	6	95.67	11.29	89.017	0.000	HS
	D.W.	6	259.17	27.49			
	W.E. 10%	6	192.83	21.99			

d.f=2

Table 2: Mean and standard deviation of C. albicansX10^2 of three mouth washes *in vivo*.

Time	Agents	No.	Mean	± SD	F	P	Description
base	CHX	6	14.33	5.12	0.658	0.532	NS
	D.W.	6	11.16	3.71			
	W.E.30%	6	13.16	5.49			
1 min	CHX	6	8.16	4.16	0.299	0.746	NS
	D.W.	6	9.66	3.50			
	W.E.30%	6	9.66	3.93			
15 min	CHX	6	4.66	2.73	2.344	0.130	NS
	D.W.	6	8.16	3.12			
	W.E.30%	6	6.50	2.50			
30 min	CHX	6	2.83	2.31	9.121	0.003	HS
	D.W.	6	9.00	3.16			
	W.E.30%	6	4.83	2.04			
1 hr	CHX	6	1.83	2.04	12.980	0.001	HS
	D.W.	6	9.83	3.18			
	W.E.30%	6	5.66	2.80			

d.f=2

Table 3: Mean and standard deviation of salivary flow rate before and after small cardamom, CHX and D.W mouthwashes.

Time	Agents	No.	Mean	± SD	F	P	Description
base	CHX	6	3.43	0.34	0.504	0.684	NS
	D.W.	6	3.30	0.33			
	W.E. 10%	6	3.45	0.50			
	W.E 30%	6	3.21	0.33			
1 min	CHX	6	3.71	0.27	1.552	0.232	NS
	D.W.	6	3.38	0.31			
	W.E. 10%	6	3.60	0.43			
	W.E. 30%	6	3.38	0.24			
15 min	CHX	6	3.90	0.23	3.147	0.048	S
	D.W.	6	3.43	0.28			
	W.E. 10%	6	3.71	0.38			
	W.E. 30%	6	3.51	0.21			
30 min	CHX	6	4.16	0.28	9.301	0.000	HS
	D.W.	6	3.40	0.21			
	W.E. 10%	6	3.83	0.27			
	W.E. 30%	6	3.68	0.24			
1 hr	CHX	6	4.11	0.27	9.051	0.001	HS
	D.W.	6	3.36	0.23			
	W.E. 10%	6	3.73	0.21			
	W.E. 30%	6	3.58	0.29			

d.f=3

Table 4: Mean and standard deviation of salivary pH before and after cardamom extracts, CHX and D.W mouth washes.

pH	Agents	No.	Mean	± SD	F	P	Description
base	CHX	6	7.14	0.10	1.114	0.367	NS
	D.W.	6	7.12	0.10			
	W.E. 10%	6	7.05	0.07			
	W.E. 30%	6	7.13	0.09			
1 min	CHX	6	7.30	0. 09	4.599	0.013	S
	D.W.	6	7.17	0.08			
	W.E. 10%	6	7.13	0.05			
	W.E. 30%	6	7.25	0.10			
15 min	CHX	6	7.42	0.09	9.252	0.000	HS
	D.W.	6	7.22	0.08			
	W.E. 10%	6	7.20	0.06			
	W.E. 30%	6	7.35	0.09			
30 min	CHX	6	7.60	0.07	34.633	0.000	HS
	D.W.	6	7.20	0.09			
	W.E. 10%	6	7.25	0.04			
	W.E. 30%	6	7.44	0.08			
1 hr	CHX	6	7.66	0.07	47.053	0.000	HS
	D.W.	6	7.16	0.09			
	W.E. 10%	6	7.21	0.05			
	W.E. 30%	6	7.39	0.09			

d.f=3