Efficacy of some antibacterial agents against Streptococcus mutans associated with tooth decay

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Abstract

Isolation of Streptococcus mutans from 79.3% of total positive culture of tooth decay cases, the presence of double isolate was the major mode of the bacterial isolates.Testing of various extracts of Punica grantum (pomegranate); red & white fruit juice, fruit crust and three of tooth pastes (signal-2, close-up and sinan) with comparison to standard antibiotics were carried out in this study. The highest diameter of inhibition zone recorded for the concentration 1000 mcg/ml of fruit crust (26 mm).The limits of MICs for pomegranate extracts are between 80-400 Mg / ml, and 200-500 Mg / ml for tooth paste concentrations.The effects of pomegranate extracts are between the limits of standard antibiotics

INTRODUCTION

Tooth decay (dental caries) is a disease of multifactorial etiology. The essential factors include: the appropriate number and species of bacteria ,the type, quantity and frequency of consumption of fermentable carbohydrates, and susceptible tooth surfaces.(Loumans, etal; 1980) . Theoretically, tooth decay can be prevented by eliminating any one of these interacting factors. Water flouridation for example, has reduced caries by about (50%) without any additional therapeutic discipline. (yagot, 1981). The great deal of evidence has been accumulated which implicates Streptococcus mutans as a major etiological agent in the initiation of enamel caries.(Jagtap & Karkera, 2000; Hamilton & Svensater, 1998). It therefore seems reasonable that topical applications of chemotherapeutic agents may offer some promise for reducing the number of or completely eliminating Streptococcus mutans and perhaps other odontopathic bacteria from the infected tooth surfaces (Chen & Burne, 1996).Clinical studies have shown that teeth are most susceptible to tooth decay during the first 2 years after eruption. On the other hand, the greatest benefit of topical fluoride has been shown to occur in young persons who have newly erupted teeth and who reside in low fluoride areas (Tichy & Novak, 1998).

One of the most promising possibilities for the control of tooth decay involves the use of combined topical applications of fluoride with chemotherapeutic agents to increase the resistance of newly erupted teeth, enhance demineralization of hypomineralized enamel, and reduce microorganisms form infected teeth (Rupf, etal., 1999; Chen, etal., 1996).

This study aimed to evaluate antibacterial activity of extracts of Punica grantum (pomegranate) and comparison with various tooth pastes and commercial antibiotics against Streptococcus mutans on teeth surfaces with tooth decay.

MATERIALS AND METHODS

SAMPLING AND CULTURING

A total of sixty oral swabs collected from buccal surfaces of the caries teeth of human subject (out patients of alshaheed qais specialized dentology center – Basrah) were plated on blood agar (Oxoid). The plates were incubated aerobically for 24 hours at 37 C and identify the bacterial types. Streptococcus mutans colonies were isolated, subcultured and characterized according to (Finegold & Baron, 1986).

The colonies of Streptococcus mutans are convex, surrounded by greenish hemolytic area(□-hemolytic streptococci) , grow at 4% NaCl, dextrane producing when grow at 10% bile salts, not hydrolyze starch.

ANTIBACTERIAL AGENTS PREPARARTIONS:

Plant extracts: we used a fruit of pomegranate Punica grantum in this study as follows:

Four types of pomegranate juice (red at pH = 6.4 and 4.6, and white at pH = 6.2 and 4.3).

Serial concentrations of fruit crust 50 – 1000 µg/ml were prepared according to (Al-Saimary & Baker, 2001).

TOOTH PASTES :

Three types of tooth pastes were used in this study: Signal-2 (Turkish), Close-up (Egyptian), and Sinan (Chinian) and using three concentrations of each tooth pastes (100, 500, 1000 mcg/ml).

ANTIBIOTICS:

Six types of commercial antibiotics (Oxoid) were used in comparison study these are: Erythromycin (15 mcg), Penicillin G (10 unit), Vancomycin (30 mcg), Cephalexin (30 mcg), Tetracyclin (30 mcg) and gentamycin (10 mcg).

MEASURING OF ANTIBACTERIAL ACTIVITY:

Agar diffusion (plate) method used to determine the growth inhibition zones (GIZ) (mm) by using Muller-Hinton Agar (Difco). 0.1 ml from 18-24 hours culture of Streptococcus mutans spreaded on M. H. A and add 5 µl of the antibacterial agents from each concentrations on the cultured surfaces.

Tube (dilution) method used to determine Minimal Inhibitory Concentration (MIC) (µg/ml or mcg/ml) by using Brain-Heart Infusion (Oxoid). MIC: is the lowest concentration of antibacterial agents that kill the bacterial growth.

RESULTS

Form sixty samples of dental caries patients, the majority of bacterial types was for Streptococcus mutans which isolated from 79.3% of total sample, while other as follows:

E. coli 27.5%, Klebsiella 37.9%, Proteus 22.4%, Pseudomanas 41.3%, Stph. Aureus 17.2% and other Streptococcus spp. 51.7%. (Figure 1)

In Table (2) the mode of isolated bacterial types are shown, the biggest mode is for double pathogenes (34.48%), followed by third (27.58%), over than three pathogenes (24.13%), and single pathogene isolated from (13.79%) from total cases.

The total number of isolates is (161).

Table (3) summerized the results of antibacterial activity of plant extracts, tooth pastes, antibiotics determined by two methods Inhibition zone, MICs.

The nearest natural red pomegranate juice is effective more than the acidic and white natural and acidic white juice not effective.

The biggest diameter recorded for 1000 mcg/ml of fruit crust was (26 mm) than followed by other concentrations. Some of tooth pastes are not effective, however, in high concentration, but other such as signal-2 and close-up give 12,10 mm respectively of inhibition zones.

Concentration 100 mcg/ml of tooth pastes are not effective on growth of Streptococcus mutans .

In comparison – The standard antibiotics give the following inhibition zones:

16 mm of erythromycin, 10 mm of penicillin, 30 mm of vancomycin, 24 mm of cephalexin, 12 mm of tetracyclin and 22 mm of gentamycin.

The MICs for the plant extracts are as follow:

100-120 mcg/ml for pH 6.4 red juice and pH 6.2 white juice

200 mcg/ml for pH 4.6 red juice

400 mcg/ml for pH 4.3 white juice

80-90 mcg/ml for fruit crust

200-220 mcg/ml for signal-2 tooth paste

250-260 mcg/ml for close-up tooth paste

500 mcg/ml for sinan tooth paste

Figure 1

Table – 2: Infectious bacterial types isolated

Mode of isolated bacterial types	No. of cases	%	No. of isolated types (161)	%
Single	8	13.79	8	4.96
Double	20	34.48	40	24.84
Third	16	27.58	48	29.81
Over than three	14	24.13	64	39.75

Figure 2

Table (3): A comparison of susceptibility for antimicrobial agents, plant extract and antibiotics against isolated from dental caries

Antibacterial agent	Inhibition zone	MIC (µg / ml)
1. Plant extract		
a. Juice		
Red juice		
pH 6.4	16	100-120
pH 4.6	10	200
White juice		
pH 6.2	10	100-120
pH 4.3	NE	400
b. Fruit crust (µg / ml)		
1000	26	
750	18	
500	14	
250	8	
100	NE	
50	NE	
MIC		80-90
2. Tooth paste		
Signal – 2 (Turkish)		
1000	12	
500	6	
100	NE	
MIC		200-220
Close – up (Iraqi)		
1000	10	
500	6	
100	NE	
MIC		250-260
Sinan (Chines)		
1000	NE	
500	NE	
100	NE	
MIC		500
3. Antibiotics		
Erythromycin (15 mcg)	16	
Penicillin G (10 Units)	10	
Vancomycin (30 mcg)	30	
Cephalexin (30 mcg)	24	
Tetracyclin (30 mcg)	12	
Gentamycin (10 mcg)	22	

DISCUSSION

Bacteria have been identified as the etiologic agents of many medically – important infections in man and animals.

Many studies demonstrated that teeth were demineralized when exposed to salivary bacteria and carbohydrates in vivo, he postulated that bacteria were the etiologic agents of dental caries, and rejected the concept that specific microorganisms could cause caries, holding instead that any and all of the acidogenic microorganisms in the mouth contributed to the process. Japtap & Karkera, 2000; (Rupf, et al., 1999)

Our results accompanied above study and results of other studies such as (Jagtap & Karkera, 2000; Schuber, et al., 1999; Chen & Burne, 1996) who evidenced that the Streptococcus mutans constitutes a majority of the total bacteria present in dental caries samples.

Other studies demonstrated that the initiation of a history of a previous Streptococcus mutans infection. In the absence of such a history, no caries occurred. The relationship of salivary concentration of Streptococcus mutans and the colonization of this microorganism on teeth has been studied in human, with an increase in Streptococcus mutans to levels of 5 X 10^4 colony-forming units CFU / ml or higher most of the surfaces of the teeth were colonized (Rupf, et al., 1999).

The results of the present study revealed that pomegranate extracts- in especially- fruit crust extract is highly effective on growth of Streptococcus mutans in comparison with other extracts and various concentrations of tooth pastes, this fact may reflect efficiency of antibacterial activity of plant extracts, and ability of bacteria to resist other antibacterial agents such as pastes and antibiotics (Schubert, et al., 1999; Tichy & Novak, 1998).

Also these results may be correlated with the aspects involving the ecological balance and interaction of oral flora with the human host may appear to make prospects for chemotherapeutic management of dental caries a difficult task, and the mechanism of action of systemic fluorides not clearly understood.

Although clinical evidence suggested that at lest part of the action of systemic fluorides against caries must be a local action on or at the surface of erupted teeth (Yagot, 1981).

CONCLUSIONS & RECOMMENDATIONS

Streptococcus mutans constitutes a majority of the bacterial types associated with dental caries.

The high percentage of isolated more is for double mode (which yielded two bacterial types in same sample).

Studied tooth pastes give a lowest effects and some of it not effective on bacterial growth.

Plant extracts (pomegranate) varied in effects, the highest effect was for 1000 mcg/ml of fruit crust extract.

The limits of MICs for pomegranate extracts are between 80-400 mcg/ml and 200-500 mcg/ml for tooth paste concentrations.

The effects of plant extracts are within the range of effects of standard antibiotics studied in this investigation.

We conclude :

More care of oral cavity and teeth- in especially-.

Using a pomegranate extract or fruit crust after eating or before sleeping with combination to tooth paste.

Change the tooth paste in serial times within 2-3 months to prevent resistance of bacteria to tooth paste.

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