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Short Communication

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

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Isolation of *Streptococcus mutans* was achieved from 79.3% of total positive culture of tooth decay cases. Testing of various extracts of *Punica grantum* (pomegranate), red and white fruit juice, fruit crust and three 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 was recorded for 1000 mcg/ml of fruit crust (26 mm). The MICs for pomegranate extracts are between 80 - 400 mg/ml, and 200 - 500 mg/ml for tooth pastes. The effects of pomegranate extracts are between the limits of standard antibiotics.

Key words: *Streptococcus mutans*, *Punica grantum*, tooth decay, pomegranate extracts, fruit crust.

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 (Youmans et al., 1980). Theoretically, tooth decay can be prevented by eliminating any one of these interacting factors. Water fluoridation 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 and Karkera, 2000; Hamilton and Svensater, 1998). It therefore seems reasonable that topical applications of chemotherapeutic agents may offer some promise for reducing the number of or completely eliminating *S. mutans* and perhaps other odontopathic bacteria from the infected tooth surfaces (Chen and 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 and 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 et al., 1999; Chen et al., 1996).

This study aimed to evaluate antibacterial activity of extracts of *Punica grantum* (pomegranate) and comparison with various tooth pastes and commercial antibiotics against *S. 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 h at 37°C and the bacterial types were identified. *S. mutans* colonies were isolated, subcultured and characterized according to (Finegold and Baron, 1986). The colonies of *S. mutans* are convex, surrounded by greenish hemolytic area (α-hemolytic streptococci), growing at 4% NaCl, dextrane producing when grown at 10% bile salts, and does not hydrolyze starch.

Plant extracts

The fruit of pomegranate *P. grantum* was used 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 and Baker, 2001).

Tooth pastes

Three types of tooth pastes were used in this study: Signal-2

Table 1. Infectious bacterial types isolated from positive culture of tooth decay cases.

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

(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 h culture of *S. mutans* was spread on Muller-Hinton Agar and 5 µl of the antibacterial agents were added 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

From sixty samples of dental caries patients, the majority of bacterial types were *S. mutans* isolated from 79.3% of total samples, while others were *Escherichia coli* (27.5%), *Klebsiella* (37.9%), *Proteus* (22.4%), *Pseudomanas* (41.3%), *Staphylococcus aureus* (17.2%) and another *Streptococcus* spp. (51.7%). In Table 1, the mode of isolated bacterial types are shown; the biggest mode is for double pathogenes (34.48%), followed by triple (27.58%), over three pathogenes (24.13%), and single pathogen isolated from (13.79%) from total cases. The total number of isolates was (161).

Table 2 summerized the results of antibacterial activity of plant extracts, tooth pastes, antibiotics determined by two methods inhibition zone and MICs. The natural red pomegranate juice is more effective than the acidic and white natural, while the acidic white juice not effective. The biggest diameter was recorded for 1000 mcg/ml of fruit crust 26 mm). Concentration of 100 mcg/ml of tooth pastes are not effective on growth of *S. mutans*. In comparison, the standard antibiotics give the following inhibition zones: 16 mm for erythromycin, 10 mm for penicillin, 30 mm for vancomycin, 24 mm for cephalexin, 12 mm for tetracyclin and 22 mm for 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

Table 2. A comparison of susceptibility for antimicrobial agents, plant extract and antibiotics against *S. mutans* 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 (Chinese)		
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	

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; and 500 mcg/ml for Sinan tooth paste.

DISCUSSION

Bacteria have been identified as the etiologic agents of many medically important infections in man and animals. Many studies have demonstrated that teeth are demineralized when exposed to salivary bacteria and carbohydrates *in vivo*. It has been postulated that bacteria were the etiologic agents of dental caries, and the concept that specific microorganisms could cause caries has been rejected, instead any and all of the acidogenic microorganisms in the mouth contributed to the process (Japtap and Karkera, 2000; Rupf et al., 1999).

Our results confirm the studies of Jagtap and Karkera (2000), Schuber et al. (1999), and Chen and Burne (1996) with evidence that *S. mutans* constitutes a majority of the total bacteria present in dental caries samples. The relationship of salivary concentration of *S. mutans* and the colonization of teeth by this microorganism has been studied in human: with an increase in *S. mutans* to levels of 5 X 10^4 CFU/ml or higher most of the surfaces of the teeth are colonized (Rupf et al., 1999).

The results of the present study revealed that pomegranate extracts, in especially, fruit crust extract is highly effective for inhibition of *S. mutans* growth in comparison with other extracts and various concentrations of tooth pastes. This fact may reflect efficiency of antibacterial activity of plant extracts (Schubert et al., 1999; Tichy and Novak, 1998).

Conclusions

S. mutans constitutes a majority of the bacterial types associated with dental caries. We recommend more care of oral cavity and teeth especially, as well as using a pomegranate extract or fruit crust after eating or before sleeping with combination to tooth paste. Also, change the tooth paste several times within 2 - 3 months is necessary to prevent resistance of bacteria to tooth paste.

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