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Inhibition of *Streptococcus mutans* Adhesion to Buccal Epithelial Cells by an Aqueous Twigs Extract of *Salvadora persica*

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Abstract

The effect of preincubation of either *Streptococcus mutans* or buccal epithelial cells (BECs) with different concentrations of aqueous *Salvadora* twigs extract (ASTE) was investigated, as well as the effect of mouth rinse with ASTE and chlorhexidine digluconate on the adhesion of bacterial cells to BEC. Inhibition (54–87%) of bacterial adhesion to preincubated BECs with ASTE was obtained compared to 47–94% adhesion inhibition to BECs when bacterial cells were preincubated with ASTE. There was a significant reduction in the adherence of bacterial cells to BECs (84%) after mouth rinse with 20% ASTE compared to 45% adhesion inhibition with chlorhexidine digluconate. The diminished adherence of *S. mutans* to BECs after exposure to various concentrations of ASTE may have clinical relevance.

Keywords: Adhesion, chlorhexidine digluconate, *S. mutans*.

Introduction

A variety of oral hygiene measures have been performed since the dawn of time. The most widely used tree twigs since early times is *meswak*, which is a stick obtained from a twigs of a plant called *Salvadora persica* (Salvadoraceae) that grows in tropical areas. Meswak chewing sticks are widely used as a traditional oral hygiene tool in several Middle East, Asian, and African countries. The periodontal status of adult habitual users of meswak chewing sticks or toothbrushes were studied (Darout et al., 2000).

The chemical analysis of meswak sticks for fluoride, calcium, phosphorus, and sili was reported by Hattab (1997). The antimicrobial properties of *Salvadora persica* extracts have been reported (Al-Bagieh et al., 1994; Al-Lafi & Ababneh, 1995; Almas, 1999).

In this study, we report the effect of an aqueous extract of *Salvadora persica* stem on the growth of an oral pathogen *Streptococcus mutans* and on the adhesion of this bacterium on buccal epithelial cells.

Materials and Methods

Organism and growth conditions

Streptococcus mutans was isolated from periodontal patients at the university clinic. The organism was grown on blood agar and on brain heart infusion medium at 37°C. Identification was carried out according to Cowan & Steel (1987) and then confirmed using API strep kit (API Laboratory Products Ltd, Basingstroke, England). Brain heart infusion broth medium containing fresh 1.25%, 2.5%, 5.0%, and 10.0% (w/v) twigs extract of *Salvadora persica* were sterilized at 121°C, 10 psi for 10 min, and pH of the medium was adjusted to 7.4. Conical flasks (250 ml) containing 100 ml medium were inoculated with 1.0 ml (10^6 cells/ml) of a log phase *S. mutans* and incubated at 37°C in a rotary shaker water bath. Growth was measured by following the absorbance at 420 nm for different intervals.

Preparation of an aqueous *Salvadora* twigs extract (ASTE)

Forty grams of *Salvadora persica* twigs (after cutting them into small pieces) were soaked in 100 ml distilled

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water for 12 h then filtered using a double layer of cheese cloth after blending in a vortex mixer at high speed. The filtrate was then centrifuged at 1000 × *g* for 10 min and passed through a 0.45-μm filter to remove any microbial contaminants.

Treatment of *S. mutans* with ASTE

The effect of ASTE on the adherence of *S. mutans* on buccal epithelial cells (BECs) was studied by incubating the bacterial cells (10^6 cells/ml) in the presence of 0%, 1.25%, 2.5%, 5.0%, and 10.0% ASTE Incubation was in a rotary shaker for 1 h at 37°C. Bacterial cells were then harvested and washed twice with Hank's balanced salt solution (HBSS). These cells were used in the adherence assay.

Preparation of BECs for adherence assay

Buccal epithelial cells were collected from six healthy students by gently rubbing the mucosal surface of the cheeks with a sterile tongue depressor. The epithelial cells were washed twice with HBSS and collected by centrifugation at 500 × *g* for 10 min to wash away saliva and other contaminating oral secretions. Cell pellet was standardized to 10^5 cells/ml.

Effect of treating BECs with ASTE

Buccal epithelial cells were collected as described above and suspended in 8 ml of HBSS. This cell suspension was divided into four equal samples of 2 ml (10^5 cells/ml). Samples were then exposed to different concentrations of ASTE (final concentrations, 1.25%, 2.5%, 5.0%, and 10%). The forth tube was used as a control. Incubation was done at 37°C for 1 h in a shaker water bath.

In another experiment, the effect of mouth rinse with ASTE (5%, 10%, and 20%) was studied using the method of Tobgi et al. (1987). BECs were collected from healthy adult students by gently rubbing the right cheek with a sterile tongue depressor, which was then agitated in 5 ml HBSS. This acted as a control for each experiment. Subsequently, the mouth was rinsed with 10 ml ASTE (5%, 10%, 20%) for 1 min, followed by a 10 ml tap-water rinse for 5 s. BECs were immediately harvested from the left cheek and suspended in 5 ml HBSS.

Also, in another experiment, the mouth was rinsed with 5 ml chlorhexidine digluconate for 1 min, and then BECs were collected and processed in the same way as with ASTE.

Adherence assay

Adherence assays were performed as described by Ghannoum et al. (1986). A mixture of equal volumes of BECs (1×10^5 cells/ml) and bacteria (1×10^6 cells/ml) from an overnight culture, was incubated at 37°C for 2 h in a rotary shaker water bath at a speed of 100 rpm and the adherence assayed microscopically. The number of bacterial cells adhering to every BEC was counted for about 50 BECs, taken at random calculation.

Results and Discussion

The effect of various concentrations of *Salvadora* twigs extract on the growth of *S. mutans* was studied as shown in Figure 1. *Salvadora* twigs extract 1. 25% exerted about 42% inhibition (Fig. 1). However, 10% ASTE resulted in 96% inhibition.

The effect of *in vitro* adherence of *S. mutans* on preincubated BECs in different concentrations of ASTE at 37°C is shown in Table 1. Preincubation of BECs with ASTE for 1 h resulted in a significant reduction (87%) of adherence of *S. mutans* to BECs even at the lowest ASTE concentration (1.25%) used; 54% inhibition was

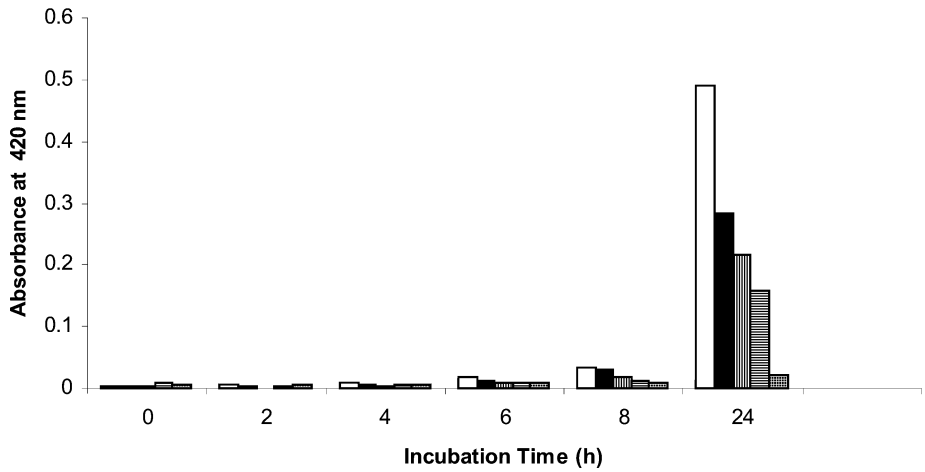

Figure 1. Effect of different concentrations of *S. persica* twigs extract on the growth of *S. mutans*: □ 0%, ■ 5%, ▥ 10%, ▤ 20%. ▦

Table 1. The effect of preincubation of human BECs with various concentrations of ASTE on adherence of *S. mutans*.[a]

ASTE (%)	Adherent bacterial cells per BEC	Reduction (%)
0.0 (control)	449	
1.25	205	54
2.5	131	70
5.0	100	77
10.0	58	87

ASTE, aqueous *Salvadora* twigs extract; BECs, buccal epithelial cells.
[a]Numbers represents an average of duplicate readings with SD 4%.

obtained as compared to control (Table 1). However, pretreatment of bacterial cells with ASTE showed a significant reduction in adhering to BECs as shown in Table 2. Preincubation of bacterial cells with 10% ASTE resulted in 94% adherence inhibition to BEC (Table 2). Pretreatment with 1.25% ASTE showed 47% inhibition (Table 2). The effect of mouth rinse with various concentrations of ASTE on the adherence of *S. mutans* to BECs is presented in Table 3. A significant reduction in bacterial adherence to BECs was observed as the concentration of ASTE used for mouth rinse was increased. The adherence of *S. mutans* to BECs, collected after 1 min of an oral rinse with 20% ASTE, was significantly reduced (84%) as shown in Table 3. Inhibition of 23% was obtained with 5% ASTE. However, oral rinse with chlorhexidine digluconate for 1 min inhibited adherence of *S. mutans* to BECs taken immediately by 45% (Table 3).

The results obtained in this study indicate that in addition to the antibacterial effects of ASTE (Fig. 1), ASTE significantly inhibited the adherence of *S. mutans* on BECs (Tables 1–3).

Meswak was found to be effective on the growth of *Staphylococcus aureus* with an minimum inhibitory concentration (MIC) value of 69 mg/100 ml (Al-Lafi & Ababneh, 1995). However, the effect of meswak on the growth of *Streptococcus mutans* and *Streptococcus faecalis* was studied by Almas (1999) using up to 50% meswak extract.

Table 2. Adherence of *S. mutans* to human BECs incubation of bacterial cells with different concentrations of ASTE.[a]

ASTE (%)	Adherent bacterial cells per BEC	Reduction (%)
0.0 (control)	279	
1.25	148	47
2.5	64	77
5.0	44	84
10.0	17	94

ASTE, aqueous *Salvadora* twigs extract; BECs, buccal epithelial cells.
[a]Numbers represents an average of duplicate readings with SD 4%.

Table 3. Adherence of *S. mutans* to human BECs collected after oral rinses with various concentrations of ASTE and chlorhexidine digluconate.[a]

ASTE (%)	Adherent bacterial cells per BEC	Reduction (%)
0.0 (control)	82	
5.0	63	23
10.0	35	57
20.0	13	84
Chlorhexidine digluconate	45	45

ASTE, aqueous *Salvadora* twigs extract; BECs, buccal epithelial cells.
[a]Numbers represents an average of duplicate readings with SD 4%.

In vivo studies concerning the periodontal status of a group of people habitual to the uses of meswak and toothbrushes showed that meswak users had significantly lower dental calculus and a lower gingival bleeding in the posterior sextants compared to the toothbrush users (Darout et al., 2000). It was also found that meswak, in addition to its effect on dental caries through its fluoride content, acts as a brush for removing dental plaque and polishing the teeth (Hattab, 1997). Aqueous extract of meswak was found to inhibit the growth of *Candida albicans* using 15% extract (Al-Bagieh et al., 1994). Extensive efforts have been made to search for an effective antiplaque agent from a variety of chemical and biological compounds (Mandel, 1988; Marsh, 1992). To date, only chlorhexidine and Listerine have gained the approval of the American Dental Association Council on Dental Therapeutics, although various adverse effects such as teeth staining and increased calculus formation were observed. Chlorhexidine was found to have a comparable activity with 10% ASTE on preventing *S. mutans* adherence on BECs (Table 3). However, preincubation of BECs with ASTE has a lower percentage reduction of *S. mutans* on these cells compared to bacterial preincubation (Tables 1 and 2). Fewer reports are available concerning the effects of antimicrobial agents from higher plants against oral pathogens (Barel et al., 1991). Further studies on the effect of ASTE on gram-negative anaerobic periodontal oral pathogen *Porphyromonas* are in progress.

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References

Al-Bagieh NH, Idowu A, Salako NO (1994): Effect of aqueous extract of miswak on the *in vitro* growth of *Candida albicans*. *Microbios 80*: 107–113.

Al-Lafi T, Ababneh H (1995): The effect of the miswak (chewing sticks) used in Jordan and the Middle East on oral bacteria. *Int Dent J 45*: 218–222.

Almas K (1999): The antimicrobial effects of extracts of *Azadirachta indica* and *Salvadora persica* chewing sticks. *Indian J Dental Res 10*: 23–26.

Barel S, Segal R, Yashphe J (1991): The antimicrobial activity of the essential oil from *Achillea fragrantissima*. *J Ethnopharmacol 33*: 187–191.

Cowan ST, Steel KJ (1987): Manual for the identification of medical bacteria. Cambridge, England: Cambridge University Press.

Darout IA, Albandar JM , Skaug N (2000): Periodontal status of adult Sudanese habitual users of miswak chewing sticks or toothbrushes. *Acta Odontol Scand 58*: 25–30.

Ghannoum MA, Burns GR, Abu El-teen K, Radwan SS (1986): Experimental evidence for the role of lipids in adherence of *Candida* spp. To human buccal epithelial cells. *Infect Immunity 54*: 189–193.

Hattab FN (1997): Meswak: the natural toothbrush. *J Clin Dentistry 8*: 125–129.

Mandel ID (1988): Chemotherapeutic agents for controlling plaque and gingivitis. *J Clin Periodontal 15*: 488–498.

Marsh PD (1992): Microbiological aspects of the chemical control of plaque and gingivitis. *J Dent Res 71*: 1431–1438.

Tobgi RS, Samaranayake IP, Macfarlane TW (1987): Adhesion of *Candida albicans* to buccal epithelial cell-sexposed to chlorhexidine gluconate. *J Med Vet Mycol 25*: 335–338.