

EFFECT OF CHA-EM THAI MOUTHWASH ON SALIVARY LEVELS OF MUTANS STREPTOCOCCI AND TOTAL IgA

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Abstract. The aim of this study was to investigate the effect of Cha-em Thai (*Albizia myriophylla*) mouthwash on the mutans streptococci (MS) and immunoglobulin A (IgA) level in saliva. Sixty-seven schoolchildren, age 6-12 years, with MS more than 1×10^5 cfu per milliliter of saliva were entered in this study. They were divided into two balanced groups according to their baseline MS counts ($\geq 10^5$ cfu/ml). Each group was randomly assigned to use either the Cha-em Thai mouthwash or the placebo mouthwash. After twice daily rinses with their mouthwash for 2 weeks, stimulated saliva were collected and analysed. The MS counts and IgA levels for the two groups were compared. Those rinsing with Cha-em Thai mouthwash showed a significant reduction in MS counts ($p < 0.05$), but the IgA levels were not different. Those rinsing with the placebo mouthwash showed no statistically significant differences in the MS counts or the IgA levels ($p > 0.05$). The results indicate that twice daily use of Cha-em Thai mouthwash can reduce the levels of MS in saliva.

INTRODUCTION

Several antimicrobial chemicals, incorporated into mouthwash, can be beneficial in the prevention of dental caries. Supplementation of mechanical brushing with effective antimicrobial mouthwash has proven beneficial in the control of plaque (Kornman, 1986a; Mandel, 1988). The greatest success has been with chlorhexidine, which is now considered the gold standard against which the other potential antiplaque agents are measured (Kornmann, 1986b; Mandel, 1988; Hearsman and Seymour, 1955). However, side effects may occur such as discoloration of the teeth or unpleasant aftertaste may occur when these chemicals are used for an extended period (Greenstein *et al*, 1986). At present, there are large numbers of mouthrinses containing antimicrobial agents available to the public but these rarely reach the rural areas. Many studies on the effect of these mouthrinses or toothpastes on salivary microbiology, plaque

and gingivitis have been done (Collaert *et al*, 1992; Rosin *et al*, 2001a,b; Meurman *et al*, 2002; Rosin *et al*, 2002).

Several medicinal plants have been recently found to have antimicrobial properties. Extracts of these plants have been added to some toothpastes and mouthwashes to control dental caries and have been extensively surveyed (Fine *et al*, 2000; Khalessi *et al*, 2004; Matsumoto *et al*, 2004; Weiss *et al*, 2004). *Albizia myriophylla Benth* (commonly known as Cha-em Thai in Thailand) is used as a substitute for licorice due to its sweet taste. This plant is widely distributed in southeastern Asian countries, such as Thailand. Its water extract has antibacterial activity against *Streptococcus mutans* ATCC 25175 (Cholticha A, unpublished data). Cha-em Thai mouthwash was prepared to use by people in rural areas. The aim of this study was to determine the efficiency of mouthwash containing water extract of Cha-em Thai on mutans streptococci counts and total IgA in saliva.

MATERIALS AND METHODS

Albizia myriophylla (Cha-em Thai) extract

The stems of Cha-em Thai were locally col-

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lected in Kanchanaburi Province, Thailand. The stems were washed, cut into small pieces and allowed to dry at room temperature for one week. It was then ground into powder at a ball mill. One hundred grams of powder was soaked in 300 ml of distilled water for 30 minutes and then boiled for 30 minutes. After cooling, the liquid extract was passed through filter paper (0.45 µm pore size) and then adjusted to a volume of 200 ml with deionized water to obtain a 50% w/v water extract. The 50% w/v extract was stored at 4°C before preparing the mouthwash.

Mouthwash preparation

From the 50% water extract of Cha-em Thai, 40% herbal mouthwash was prepared by adding menthol, glycerine, sodium benzoate and deionized water. A placebo mouthwash was also prepared similarly except there was no Cha-em Thai extract present.

Experimental design and subject selection

Sixty-seven schoolchildren, age 6-12 years, in Kanchanaburi Province with salivary MS counts $\geq 1 \times 10^5$ cfu/ml saliva were entered in this study. They were divided into 2 balanced groups according to their baseline levels of salivary MS counts ($\geq 10^5$ cfu/ml). Each group was randomly assigned to use either Cha-em Thai mouthwash or placebo mouthwash. The subjects were asked to rinse with 10 ml of their assigned mouthwash for 1 minute twice daily for 2 weeks. At baseline and after 2 weeks mouthrinsing, stimulated saliva were collected and analysed for MS counts and total IgA.

The determination of salivary levels of MS and total IgA

Paraffin wax stimulated saliva was collected from subjects in sterile cups. One milliliter of saliva was pipetted into a small sterile vial and kept in dry ice until sent to the microbiological laboratory to determine the total IgA levels. They were stored at -20°C prior to assay for total IgA.

The salivary levels of MS were obtained immediately after saliva sample collection using a modified micromethod of Westergren and Grasse (1978). MSB agar was used as selective media for MS counts. Twenty-five microliters of 10^{-1} and 10^{-2} dilutions of saliva were spotted onto MSB agar and incubating for 2 days at

37°C. MS colonies were counted and expressed as cfu/ml saliva.

The total IgA levels in saliva were measured by solid-phase ELISA, performed in 96-well flat-bottomed plates (maxisorp micro-titer plates Nune, Roskilde, Denmark). Saliva samples were centrifuged at 10,000g at 4°C for 10 minutes. The clear supernatants were collected and frozen at -20°C until used. The Maxisorp micro-titer plate was coated with monoclonal antibody against human secretory component (IgA). One hundred microliters of saliva samples were applied to individual wells and incubated for 1.5 hours at 37°C. After washing, 100 µl of biotin-conjugated anti-human IgA antibody was added and incubated for 1 hour at 37°C, then the plate was rewashed thoroughly. One hundred microliters per well of avidin-peroxidase conjugate was then added and incubated at 37°C for another hour. The unbound enzyme conjugate was removed by washing with washing buffer. The antigen-antibody complex was visualized by adding 100 µl/well of substrate (3, 3', 5, 5'-tetramethylbenzidine - TMB) and incubated in the dark at room temperature for 30-45 minutes to allow the color to develop vividly. The enzymatic reaction was finally terminated by the addition of 50 µl of 0.5M H₂SO₄. The color was measured at a wavelength of 450 nm using a micro-titer plate spectrophotometer (BIO-TEX MQX 200, BIO-TEX Instruments, Inc, Highland Park, Vermont, USA). The quantity of total IgA was expressed in milligrams/milliliter of saliva (mg/ml).

Statistical analysis

A log transformation of MS counts (cfu/ml saliva) was carried out. Within group comparison of MS counts and IgA in saliva were calculated by Wilcoxon signed rank test and paired *t*-test respectively.

RESULTS

Sixty-seven schoolchildren with a mean MS count of $\geq 1 \times 10^5$ cfu/ml saliva or ≥ 5.0 log cfu/ml saliva, were divided into 2 groups: Cha-em Thai mouthwash and placebo groups. The Cha-em Thai mouthwash group had a mean MS count of 5.636 log cfu/ml and a mean salivary IgA of

Table 1

Comparison of salivary MS counts and IgA levels before and after Cha-em Thai mouthwash.

Observed variable (N=36)	Statistical calculation	Base line	2 weeks	Within group comparison	p-value
MS count(log cfu/ml)	Mean	5.636 (0.287)	3.750 (2.430)	Normality test failed	-
	Median	5.691	4.920	Wilcoxon signed rank test	p<0.05*
IgA (mg/ml)	Mean	0.372 (0.71)	0.327 (0.037)	Paired t-test	p>0.05
	Median	0.375	0.300	-	-

*Statistically significant difference, p<0.05

Table 2

Comparison of the salivary MS counts and IgA levels before and after placebo mouthwash.

Observed variable (N=36)	Statistical calculation	Base line	2 weeks	Within group comparison	p-value
MS count(log cfu/ml)	Mean	5.627 (0.353)	4.390 (2.267)	Normality test failed	-
	Median	5.678	5.283	Wilcoxon signed rank test	p>0.05
IgA (mg/ml)	Mean	0.351 (0.065)	0.331 (0.038)	Paired t-test	p>0.05
	Median	0.34	0.330	-	-

0.372 mg/ml at baseline, while the placebo group had a mean MS count of 5.627 log cfu/ml and a mean salivary IgA of 0.351 mg/ml at baseline. Comparison among the groups for MS counts and IgA in saliva before and after Cha-em Thai mouthwash is presented in Table 1. A significant reduction in MS count (p<0.05) was found, but no significant difference in IgA levels was seen (p>0.05).

The comparison among the placebo group of salivary MS counts and IgA levels is shown in Table 2. After 2 weeks of placebo mouthwash, no significant differences were found for either the MS counts or the IgA levels in the saliva (p>0.05).

DISCUSSION

This study was designed to evaluate the short-term effect of 40% Cha-em Thai mouthwash compared to placebo control. The 40% aqueous Cha-em Thai solution showed a significant reduction in mean salivary MS counts after 2 weeks use but did not affect the total salivary IgA. In Thai folk medicine, Cha-em Thai is used as licorice due to its sweet taste.

Yoshikawa *et al* (2002) isolated five new triterpene saponins from the stem of Cha-em Thai collected in Kanchanaburi Province, Thailand. This study also used the stem of Cha-em Thai collected from the same province to prepare 40% aqueous mouthwash and was studied in the same Province to investigate the efficiency of this mouthwash in schoolchildren. Aqueous Cha-em Thai mouthwash significantly reduced the amount of mutans streptococci from 5.6 log cfu/ml saliva to 3.7 after 2 weeks (p<0.05), which was more effective than placebo. This observation confirms that Cha-em Thai mouthwash can be used to lower salivary MS in schoolchildren. The levels of total IgA in saliva for both groups were not affected after 2 weeks of use. Koga-Ito *et al* (2004) reported a correlation among mutans streptococci counts, dental caries and IgA in saliva, but no correlation between mutans streptococci counts and anti-streptococcus mutans IgA levels were found. Camling *et al* (1987) showed that IgA to *Streptococcus mutans* did not reflect the quantity of mutans streptococci at the moment of saliva collection. These study results are similar to those studies. Total IgA levels in saliva were

nearly the same, even if the mutans streptococci levels were significantly different ($p < 0.05$). Our results show that total IgA levels in saliva do not reflect the salivary mutans streptococci concentration and Cha-em Thai mouthwash does not alter salivary levels of total IgA.

Due to the sweet taste of Cha-em Thai, Yoshikawa *et al* (2002) tested the sweetness relative to sucrose by a human sensory panel. They found that Albiziasaponin A and B have a potent sweetness intensity relative to sucrose (600 times). Our aqueous Cha-em Thai mouthwash preparation has a good taste compared to chlorhexidine. The effectiveness in lowering mutans streptococci suggests that Cha-em Thai mouthwash can be used short-term to reduce salivary levels of mutans streptococci in high caries-risk schoolchildren.

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