World Applied Sciences Journal 15 (6): 899-903, 2011
ISSN 1818-4952

A Comparative Clinical Study to Assess the Immediate Antimicrobial Effect of Miswak and Toothbrush on Cariogenic Bacteria

[1]Padma K. Bhat, [1]Amit Kumar and [2]Soumik Sarkar

[1]Department of Public Health Dentistry,
Rajarajeswari Dental College and Hospital, Bangalore, Karnataka, India
[2]Department of Microbiology and Biotechnology, Al-Ameen Arts,
Science and Commerce College, Bangalore, Karnataka, India

Abstract: *Objectives:* Assessment of immediate antimicrobial effect of miswak (chewing stick) and miswak extract as compared to toothbrush and saline on cariogenic bacteria like Streptococcus mutants and Lactobacillus. *Materials and methods:* The study was conducted clinically using participants' saliva and measuring the effect of miswak, miswak extract, toothbrush and normal saline on Streptococcus mutans and lactobacilli. Forty dental subjects aged 18-25 years were included in the study. For this study, 50% of miswak extract (solution) was used. The saliva samples were analysed for the presence of Streptococcus mutants and Lactobacillus by serial dilution technique in Mitis salivarius agar and Rogosa agar plates respectively. *Results:* It was found that miswak and its extract had very significant detrimental effect on both the dental caries causing micro-organisms at the tested conditions. Both of these methods had shown significant reduction of microbial count as compared to toothbrush and saline in the present study. In case of gender comparison, the reduction of microbial count in females was more for both the cariogenic bacteria as compared to number of males studied. *Conclusions:* This study proved the potential beneficial effect of miswak and miswak extract in modern day's oral hygiene practice.

Key words: Dental caries · Cariogenic bacteria · Miswak · Antimicrobial activity

INTRODUCTION

The chewing stick, miswak, is used in many developing countries as a traditional means for oral hygiene. It is prepared from the roots, twigs and stem of Salvadora persica, which plays a role in the promotion of oral hygiene [1]. The World Health Organization (WHO) has also recommended and encouraged the use of these sticks as an effective tool for oral hygiene [2]. The value of chewing sticks is believed to be in their mechanical cleansing action. However, the use of miswak has also been reported to inhibit the formation of dental plaque chemically and exert antimicrobial effect against many oral bacteria [3]. In vitro studies have demonstrated that aqueous extracts of miswak have growth-inhibitory effects on several micro-organisms [3-5]. Hence in the present study, an attempt was made to evaluate the immediate antimicrobial effect of miswak and its extract on the cariogenic bacteria like Streptococcus mutants and Lactobacillus and to compare it with toothbrush and saline.

MATERIALS AND METHODS

Preparation of Miswak Extract: A sample of the most commonly used chewing sticks from Miswak trees was collected from local market. The fresh miswak was cut into small pieces and allowed to dry at room temperature for couple of days. Then it was ground to powder. Successive 10 g quantity was put into sterile screw-capped bottle to which 100 ml of sterile deionized distilled water was added. The extract was allowed to soak for 48 hours at 4°C and then centrifuged at 2000 rpm for 15 minutes. The supernatant was passed through filter paper (0.45 µm pore size) and the extract was prepared at 50 % concentration. The extract was stored at 4°C and used within one week.

Corresponding Author: Dr. Padma K. Bhat, # 549, 12th Cross, Ideal Homes, Rajarajeswari Nagar, City Bangalore, Pin code: 560098, India. Cell: +9886580298.

Selection of Subject: Forty dental students (20 boys and 20 girls), aged between 18-25 years age, were selected from the Rajarajeswari Dental College and Hospital, Bangalore. Informed consent was taken from the participants and ethical clearance was obtained from the institutional ethical committee.

The students selected, were with no systemic diseases and not having any caries experience. During last two weeks before the study, they had not used any antibiotic and/or antiseptic mouthwash.

These students were divided into four groups with 10 in each (5 boys and 5 girls) group.

Group I- Miswak: Forty students were asked to use fresh miswak for 10 minutes in clinic. Saliva samples (2 ml) were collected before and after the use of the miswak by expectorating into a sterile glass tube.

Group II-50 % Miswak Extract: Forty students were asked to rinse the mouth with 50% miswak extract for 10 minutes. Saliva samples (2 ml) were collected before and after the use of miswak extract solution.

Group III-Toothbrush: Forty students were asked to brush their teeth with a new toothbrush for 10 minutes. Saliva samples (2 ml) were collected before and after the tooth brushing.

Group IV-Saline (Control): Forty students were asked to rinse the mouth with normal saline (5 ml each) for 10 minutes. Saliva samples were collected before and after the use of saline.

Collection of Saliva: The students of each group were asked to chew a sterilized rubber band for one minute and allowed to spit the saliva, not the rubber band. The process of chewing was carried out up to 3 minutes and the saliva was collected in a pre-sterilized vials containing physiological saline solution for further microbial analysis.

Microbial Analysis: The saliva samples were analysed for the presence of Streptococcus mutants and Lactobacillus by serial dilution technique in Mitis salivarius agar and Rogosa agar plates respectively. For both the organisms, same dilutions (x 10^3) were used. Lactobacillus plates were incubated at 37±2°C for 24 to 48 hours, where as Streptococcus mutants plates were incubated in anaerobic jar for 3 to 5 days.

Data Analysis: Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean±SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. Student t test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups (Inter group analysis) on metric parameters, Student t test (two tailed, dependent) has been used to find the significance of study parameters on continuous scale within each group. Analysis of variance (ANOVA) has been used to find the significance of study parameters between three or more groups of subjects. The Statistical software namely SPSS V15.0 software was used for the analysis of the data.

RESULTS

The results showed that there was marked reduction in both the cariogenic bacteria that is Streptococcus mutant and Lactobacillus after using different agents used in the present study, that is, miswak chewing stick, 50% miswak extract, toothbrush and saline.

In case of Streptococcus mutants, there was significant reduction, ($p< 0.001$) in all the four groups. Group 1-miswak chewing stick, effect size (8.73) and Group 2 - 50% miswak extract, effect size (7.81) were more than 50% compared to Group 3 - Toothbrush, effect size (3.16) and Group 4 - Saline, effect size (2.51), which shows that reduction in Group 1 and Group 2 is more significant than Group 3 and Group 4. Among four groups maximum reduction was seen in Group 1- miswak chewing stick (Table 1). Statistical analysis of the effect of these treatment on gender basis had shown that reduction of Streptococcus mutants was more in females in Group 1- miswak chewing stick (Delta value=19.40±1.67) and Group 2-50% miswak extract (Delta value=17.80±2.28) as compared to Males in Group 1- miswak chewing stick (Delta value=17.80±2.28) and Group 2- 50% miswak extract (Delta value=15.80±1.64) whereas in Group 3- Toothbrush and Group 4- Saline, reduction was more in males than females (Fig. 1).

In case of Lactobacillus, the same trend was noticed. There was significant reduction ($p<0.001$) of Lactobacillus in all the four groups. Group 1-miswak chewing stick, effect size (7.41) and Group 2 - 50% miswak extract, effect size (3.68), were more than 50% compared to values of Group 3-Toothbrush, effect size (1.96) and Group 4-Saline, effect size (2.39). This shows that reduction in Group 1 and Group 2 is more significant than Group 3 and Group 4. Overall, among four groups maximum reduction was seen in Group 1- miswak chewing sick (Table 2). Similar to

Table 1: Evaluation of Streptococcus mutans CFU (colony forming unit) (at × 10^3) count in four different groups

S. Mutans Count	Before	After	P. Value	Delta	Effect Size
Group I	23.90±2.28	5.30±1.49	<0.001**	18.00±2.06	8.73
Group II	28.70±1.77	11.90±2.18	<0.001**	16.80±2.15	7.81
Group III	26.10±2.08	18.60±1.84	<0.001**	7.50±2.37	3.16
Group IV	26.40±1.65	21.10±1.85	<0.001**	5.30±2.11	2.51

Delta=difference of after-before

Table 2: Evaluation of Lactobacillus CFU (colony forming unit) count (at × 10^3) in four different groups

Lactobacillus Count	Before	after	P Value	Delta	Effect Size
Group I	26.80±3.26	6.20±1.39	<0.001**	20.60±2.78	7.41
Group II	25.00±2.83	11.00±2.00	<0.001**	14.00±3.80	3.68
Group III	23.80±1.81	19.10±2.73	<0.001**	4.70±2.40	1.96
Group IV	25.40±3.68	21.00±3.56	<0.001**	4.40±1.84	2.39

Delta=difference of after-before

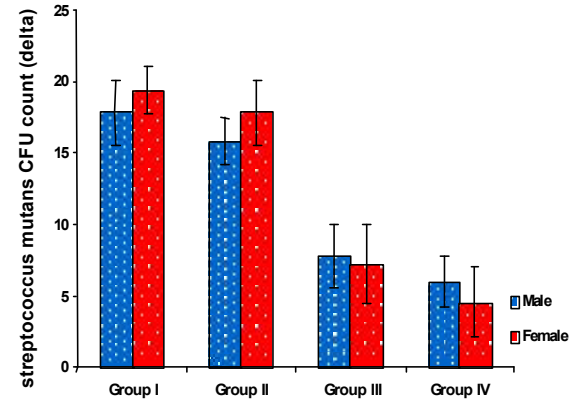

Fig. 1: Comparison of difference (Post-Pre) of Streptococcus mutans CFU (colony forming unit) count (at × 103) in males and females

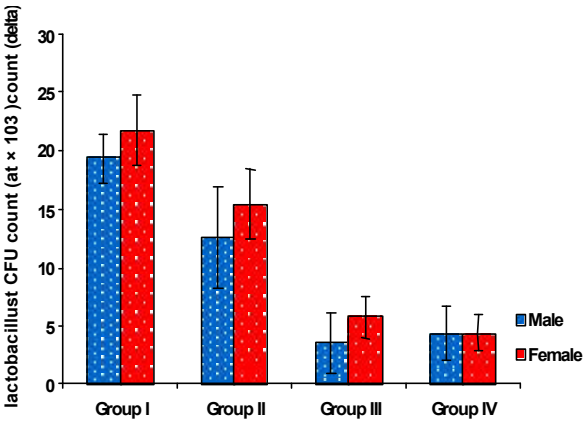

Fig. 2: Comparison of difference (Post-Pre) of Lactobacillus CFU (colony forming unit) count (at × 103) in males and females

reduction in Streptococcus mutans count, the reduction of Lactobacillus count was more in females as compared to males studied (Fig. 2).

DISCUSSION

Chewing sticks may be as effective as toothbrushes in the mechanical removal of plaque [6-9], but this evidence is not conclusive [10]. Al-lafi and Ababneh [3] investigated antibacterial effects of Miswak and showed that derivatives of these sticks have strong effects on the growth of cariogenic bacteria. Olsson [11] reported that chewing sticks are more effective in decreasing dental caries than tooth-brush. The present study shows significant reduction in both Streptococcus mutans and Lactobacillus counts in all the groups, but the efficacy was higher in miswak and its extract as compared to toothbrush and saline. In case of gender comparison, it was found that the reduction of microbial count in females was more for both the cariogenic bacteria as compared to number of males studied. The effect may be due to some biologically active compounds present in miswak. It contains trimethylamine, salvadorine chlorides, fluoride, silica, sulphur, vitamin C and small quantities of tannins, saponins flavenoids and sterols [12]. The therapeutic and prophylactic effects of miswak may be due to mechanical cleaning, the potential release of biologically active chemicals when used or as a combination of both [13]. As the miswak is often left in the mouth for some time before cleaning; the enhanced salivation may also promote better cleansing and thus improve the maintenance of oral health [10].

Darout IA *et al.* [14] assessed the salivary levels of 25 oral bacteria in relation to periodontal status and experience of dental caries and compared the levels of these bacteria between habitual miswak and toothbrush users in adult Sudanese subjects. It was found that there were significantly higher levels of streptococcus mutans and low level of lactobacillus in the miswak than in the toothbrush group whereas in the present study there was

greater reduction of both the bacteria in miswak as compared to toothbrush. Salehi P. *et al.* [15] had shown that Salvadora persica in the form of mouth wash had reduced the number of Streptococcus mutants colonies which is comparable to present study. Wolinsky *et al.* [16] showed that miswak decreases the ability of some Streptococcus to colonize on teeth surfaces where as our study shows reduction in salivary level of Streptococcus. A study conducted by Almas K *et al.* [13], showed that the reduction of Streptococcus mutans was significantly greater using miswak and its extract in comparison to tooth brushing and there was no significant difference for Lactobacilli reduction where as in our study, there was significant reduction for both the bacteria using miswak and its extract as compared to toothbrush. Sofrata AH *et al.* [17] assessed antibacterial effect of miswak against oral microorganisms associated with periodontitis and caries. A reduction in the level of streptococcus mutans was found, which is comparable to results of our study. Darmani H *et al.* [18] examined the effects of aqueous extracts of miswak on the growth of the various cariogenic microorganisms including Streptococcus mutans. The result showed inhibition in growth of Streptococcus mutans which is similar to our result. Almas K *et al.* [19] had found the antimicrobial effect of miswak extract in vitro on streptococcus mutans which shows similar results to present study. Aqueous and methanol extracts of Salvadora persica were investigated by Firas *et al.* [20] for its antimicrobial activities against seven isolated oral pathogens. The aqueous extract inhibited all isolated microorganisms, especially the Streptococcus mutans and was more efficient than the methanol extract, which was resisted by Lactobacillus whereas our study shows significant reduction in both the bacteria by miswak extract.

CONCLUSIONS

The result of this clinical study showed higher efficacy of miswak and 50% miswak extract in reduction of cariogenic bacteria as compared to conventional toothbrush. Since miswak is inexpensive, readily available, contains medicinal properties and is available in most rural areas of the developing countries, can be an effective tool in preventing oral diseases. Furthermore, the miswak extract would be acceptable culturally for oral rinsing purposes, in countries where this is used as oral hygiene method. A traditional practice so common in our country should be thoroughly investigated further on modern scientific patterns.

REFERENCES

1. Consensus Statement on Oral Hygiene, 2000. The significance of removing dental plaque mechanically every day. International Dental J., 52: 139.
2. World Health Organisation, 1984. Prevention methods and programmes for oral health. Report of a WHO Expert Committee Technical Report Series, 713, Geneva.
3. Al-Lafi, T. and H. Ababneh, 1995. The effect of the extract of the miswak (chewing stick) used in Jordan and the Middle East on oral bacteria. International Dental J., 45: 218-222.
4. Homer, K.A., F. Manji and D. Beighton, 1992. Inhibition of peptidase and glycosidase activities of Porphyromonas gingivalis, Bacteroides intermedius and Treponema denticola by plant extracts. J. Clinical Periodontol., 19: 305-310.
5. Almas, K. and N.H. Al-Bagieh, 1990. The antimicrobial effects of bark and pulp extracts of miswak, Salvadora persica. Biomedical Letters, 60: 71-75.
6. Almas, K., 2001. The antimicrobial effects of seven different types of Asian chewing sticks. Odontostomatol Trop, 24: 17-20.
7. Al-Otaibi, M., M. Al-Harthy, B. Soder, A. Gustafsson and B. Angmar-Mansson, 2003. Comparative effect of chewing sticks and toothbrushing on plaque removal and gingival health. Oral Health Prev Dent, 1: 301-307.
8. Darout, I.A., J.M. Albandar and N. Skaug, 2000. Periodontal status of adult Sudanese habitual users of miswak chewing sticks or toothbrushes. Acta Odontol Scand, 58: 25-30.
9. Gazi, M., T. Saini, N. Ashri and A. Lambourne, 1990. Miswak chewing stick versus conventional toothbrush as an oral hygiene aid. Clin Prev Dent, 12: 19-23.
10. Wu, C.D., I.A. Darout and N. Skaug, 2001. Chewing sticks: timeless natural toothbrushes for oral cleansing. J. Periodontal Res., 36: 275-284.
11. Olsson, B., 1978. Efficiency of traditional chewing sticks in oral hygiene programs among Ethiopian School- children. Community Dentistry and Oral Epidemiol., 6: 105-109.
12. Akhtar, M.S. and M. Ajmal, 1981. Significance of chewing sticks (miswaks) in oral hygiene from a pharmacological view point. J. Pak. Med. Assoc., 4: 89-95.

13. Almas, K. and Z. Al-Zeid, 2004. The immediate antimicrobial effect of tooth brush and Miswak on cariogenic bacteria. A clinical study. J. Conemp. Dent. Pract, 5:105-114.
14. Darout, I.A., J.M. Albandar, A. Skaug and R.W. Ali, 2002. Salivary microbial in relation to periodontal status, experience of caries and Miswak use in Sudanese adults. J. Clin. Periodontal, 29: 411-420.
15. Salehi, P. and M. Danaie Sh, 2006. Comparison of the antibacterial effects of persica mouthwash with chlorohexidine on Streptococcus mutans in orthodontic patients. DARU, 14(4): 178-182.
16. Wolinsky, L.E., S. Mania, S. Nachnani and S. Ling, 1996. The inhibiting effect of aqueous Azadirechta indica (Neem) extract upon bacterial properties influencing in vitro plaque formation. J. Dental Res., 75(2): 816- 822.
17. Sofrata, A.H., R.L. Claesson, P.K. Lingström and A.K. Gustafsson, 2008. Strong antibacterial effect of miswak against oral microorganisms associated with periodontitis and caries. J Periodontol, 79(8): 1474-9.
18. Darmani, H., T. Nusayr and A.S. Al-Hiyasat, 2006. Effects of extracts of miswak and derum on proliferation of Balb/C 3T3 fibroblasts and viability of cariogenic bacteria. Int. J. Dent Hygiene, 4: 62-66.
19. Almas, K., N. Skaug and I. Ahmad, 2005. An in vitro antimicrobial comparison of miswak extract with commercially available non-alcohol mouthrinses Int. J. Dent. Hygiene, 3: 18-24.
20. Firas, A., AL-Bayati, D. Khudir and Sulaiman, 2008. In vitro antimicrobial activity of salvadora persica L. extracts against some isolated oral pathogens in Iraq. Turk. J. Biol., 32: 57-62.