Assessment of immediate antimicrobial effect of miswak extract and toothbrush on cariogenic bacteria – A clinical study

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Abstract:
Objectives: Assessment of immediate antimicrobial effect of miswak (Salvadora persica) extract as compared to toothbrush and saline on cariogenic bacteria like Streptococcus mutans and Lactobacillus. **Methods:** The study was conducted clinically using participants' saliva and measuring the effect of miswak extract, toothbrush, and normal saline on Streptococcus mutans and lactobacilli. Thirty dental subjects aged 18-25 years were included in the study. For this study, 50% of miswak extract 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 extract had very significant detrimental effect on both the dental caries causing micro-organisms at the tested conditions. It 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 bacterias as compared to number of males studied. **Conclusions:** This study proved the potential beneficial effect of miswak extract in oral hygiene practice.
Key words: Dental caries, cariogenic, bacteria, antimicrobial.

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Introduction:

Miswak is the most widely used chewing stick, which is prepared from the roots or twigs of Salvadora persica, and is used in middle-eastern and eastern African cultures.[1] Various components of Salvadora persica have been reported to have beneficial biological properties, including significant antibacterial and antifungal activity.[2-4] Furthermore, extracts from miswak are reported to be effective against some periodontal pathogens and other bacteria that are important during development of dental plaque.[5-8] Despite the wide use of miswak, information on the immediate antimicrobial effect of its extract on cariogenic bacteria are still scant. Thus, the present study aimed at investigating the immediate antimicrobial effect of miswak extract on 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 de-ionized 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.[3]

Selection of subject:

Thirty dental students (15 boys and 15 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 selected students were with no systemic diseases and not having any caries experience and had not used any antibiotic and antiseptic mouthwash during last two weeks before the study. These students were divided into three groups with 10 in each (5 boys and 5 girls) group.

Group I- 50 % Miswak Extract

Ten 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 II- Toothbrush

Ten 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 III- Saline (Control)

Ten 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 minutes 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 groups of subjects. The Statistical software namely SPSS [Statistical Package for the Social Sciences V15.0 © (Chicago, USA)] 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 mutans and Lactobacillus after using different agents used in the present study, that is, 50% miswak extract, toothbrush and saline.

In case of Streptococcus mutans, there was significant reduction, (P<0.001**) in all the three groups. Group I – 50% miswak extract , effect size (7.81) was more than 50% compared to Group II – Toothbrush, effect size (3.16) and Group III – Saline, effect size (2.51), which shows that reduction in Group I is more significant than Group II and Group III. Among three groups maximum reduction was seen in Group I- miswak extract. When the Streptococcus mutans count was compared among the different groups, before and after the use of materials, it was found to be statistically significant (P<0.001**) (Table 1).

It was found that reduction of Streptococcus mutans was more in females in Group I-50% miswak extract (Delta value = 17.80±2.28) as compared to males (Delta value =15.80±1.64) whereas in Group II- Toothbrush and Group III- Saline, reduction was more in males than females. When the reduction in Streptococcus mutans was compared between different groups among males and females, it was found to be statistically significant (P<0.001**) (Figure 1).

In case of Lactobacillus, the same trend was noticed. There was significant reduction (P<0.001**) of Lactobacillus in all the three groups. Group I- 50% miswak extract, effect size (3.68), was more than 50% compared to values of Group II – Toothbrush, effect size (1.96) and Group III – Saline, effect size (2.39). This shows that reduction in Group I is more significant than Group II and Group III. Overall, among three groups maximum reduction was seen in Group I- miswak extract. When the lactobacillus count was compared among the different groups before and after the use of materials, it was found to be statistically significant (P<0.001**) (Table 2).

It was found that reduction of Lactobacillus was more in females in Group I- 50% miswak extract (Delta value = 15.40±2.97) as compared to males (Delta value =12.60±4.34). In Group II- Toothbrush, there was more reduction in females (Delta value =5.80±1.79) as compared to males (Delta value

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

GROUPS	S. MUTANS COUNT BEFORE	S. MUTANS COUNT AFTER	p VALUE	DELTA	EFFECT SIZE
Group I	28.70±1.77	11.90±2.18	<0.001**	16.80±2.15	7.81
Group II	26.10±2.08	18.60±1.84	<0.001**	7.50±2.37	3.16
Group III	26.40±1.65	21.10±1.85	<0.001**	5.30±2.11	2.51
p value	<0.001**	<0.001**			

Delta=difference of after-before
Student t test (two tailed, independent), Analysis of variance (ANOVA)
P< 0.05, statistically significant

Figure 1: Comparison of difference (Post-Pre) of Streptococcus mutans CFU (colony forming unit) count (at × 10^3) in males and females

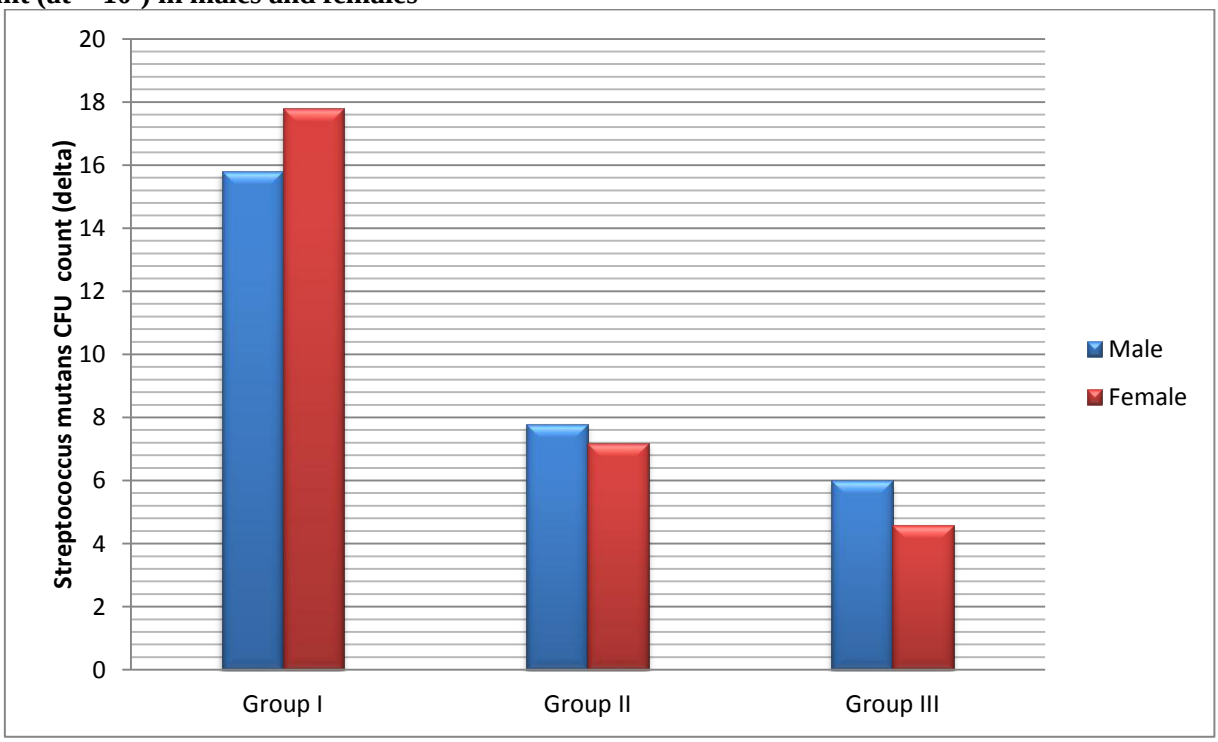
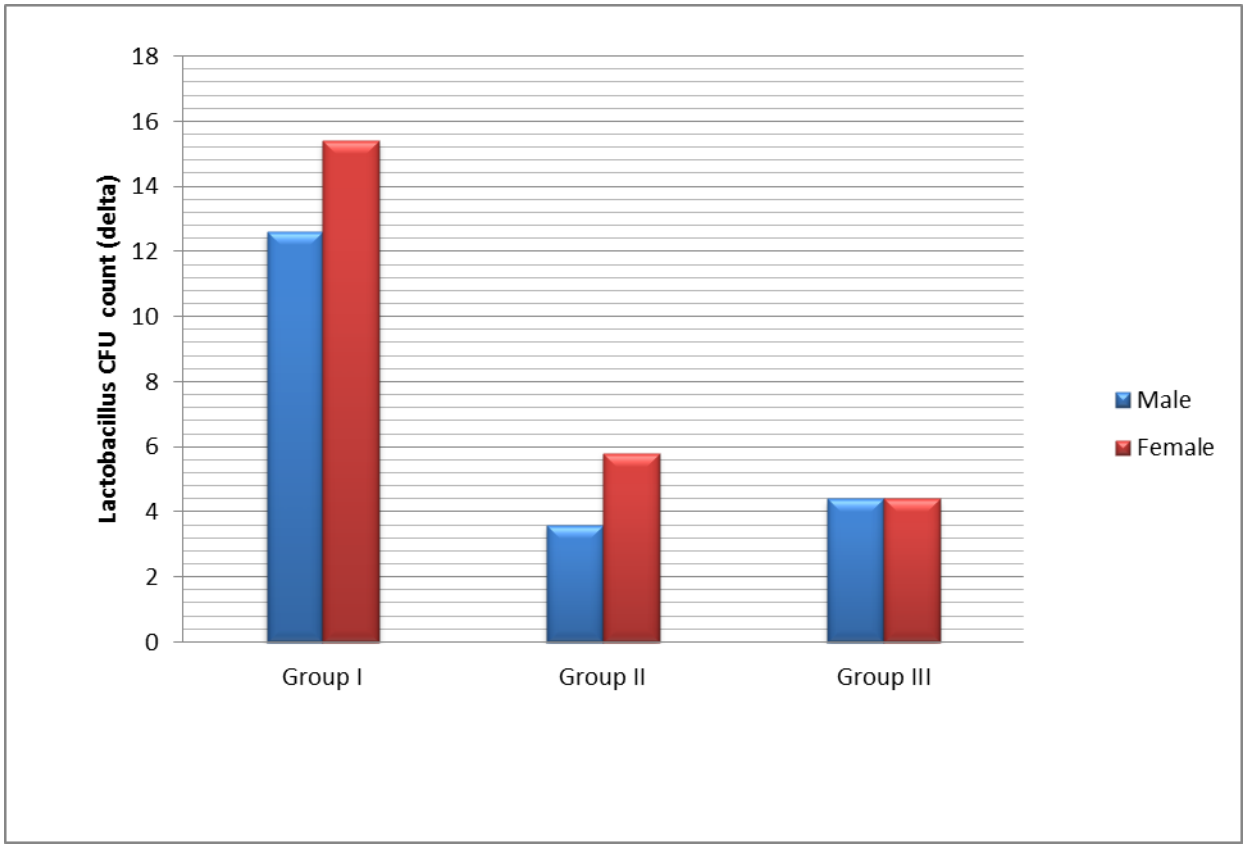


Table 2: Evaluation of Lactobacillus CFU (colony forming unit) count (at × 10^3) in three different groups

GROUPS	LACTOBACILLUS COUNT BEFORE	LACTOBACILLUS COUNT AFTER	p VALUE	DELTA	EFFECT SIZE
Group I	25.00±2.83	11.00±2.00	<0.001**	14.00±3.80	3.68
Group II	23.80±1.81	19.10±2.73	<0.001**	4.70±2.40	1.96
Group III	25.40±3.68	21.00±3.56	<0.001**	4.40±1.84	2.39
p value	<0.001**	<0.001**			

Delta=difference of after-before
Student t test (two tailed, independent), Analysis of variance (ANOVA)
P< 0.05, statistically significant

Figure 2: Comparison of difference (Post-Pre) of Lactobacillus CFU (colony forming unit) count (at × 10^3) in males and females



=3.60±2.61) and in Group III- Saline, reduction was almost similar in both males and females. When the reduction in lactobacillus was compared between different groups among males and females, it was found to be statistically significant (P<0.001**) (Figure 2).

Discussion:

The present study shows significant reduction in both Streptococcus mutans and Lactobacillus counts in all the groups, but the efficacy was higher in miswak 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. A limitation which can be considered is that the study has been done on small sample-size. The antimicrobial effect may be due to biologically active compounds present in miswak like trimethylamine, salvadorine chlorides, fluoride, silica, sulphur, vitamin C and small quantities of tannins, saponins flavenoids and sterols.[9] Almas and Al-Bagieh[10] found that aqueous extracts of Salvadora persica bark, the pulp as well as the whole miswak, were effective against various bacteria including S. mutans. Dorner[11] speculated that the high amount of NaCl, KCl, trimethylamine and sulphur-containing organic substances (salvadourea and salvadorine) in miswak might somehow be responsible for the observed antibacterial and gum-stimulating effects.[12] Furthermore, a study by Darout et al.[13] showed that aqueous miswak extracts contained potential antimicrobial anionic components in addition to chloride and sulphate, which were thiocyanate and nitrate. They hypothesized that thiocyanate leaching out from miswak, while in the oral cavity, may lead to an elevated level of salivary thiocyanate. This, in turn, may enhance the efficacy of the salivary hydrogen peroxide–peroxidase-thiocyanate system, a known antimicrobial component of human saliva.[14]

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. A study conducted by Almas K et al. [16], showed that the reduction of Streptococcus mutans was significantly greater using miswak 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 extract as compared to toothbrush. Darmani H et al.[17] 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.[18] 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.[19] 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. Al-Bayaty FH et al[20], Shingare P et al[21] and Masoumeh K et al[22] had also found the miswak extract as an effective antimicrobial agent which is comparable to our study results.

Conclusions:

The result of this clinical study showed higher efficacy of 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.

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