Original Article

Oral Manifestations and Microbiological Effects of Miswak and Toothbrush Users in a Sample of People of Kirkuk City

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Abstract

Objectives: To assess and compare the oral hygiene, gingival conditions, and salivary microbiota levels among a group of people in Kirkuk city who used miswak or toothbrush regularly for their routine daily oral hygiene.

Methods: Sixty male subjects with the age range of 25-65 (mean 35.2), 30 Miswak, and 30 Toothbrush users were enrolled in the study. The oral manifestations were assessed clinically using three different indices, while the microbial effects have been studied simplified oral hygiene index (OHI-S), gingival index (GI), and decayed, missing, filled teeth index (DMFT) by determining the level of selected bacterial species in the collected saliva samples from all participants.

Results: It showed that all three indices (OHI-S, GI, and DMFT) were significantly higher in Miswak users than in toothbrush users. There were significantly higher pathogenic microorganisms (E.coli, Candida albicans, Klebsiella) in the saliva sample of Miswak used group than in the toothbrush group. On the other hand, there were no significant differences between the two groups regarding (S.pyogenes, Lactobacillus, and Staph aureus). Also, gum recession and tooth wear (Abrasion) were significantly higher in Miswak users than in toothbrush users (P=0.01),(P=0.001), respectively.

Conclusions: This study demonstrated that the Miswak appears less efficient than tooth brushing to improve oral hygiene and periodontal conditions. Moreover, the antimicrobial agents contained in the Miswak offer no advantages over tooth brushing with toothpaste.

Keywords: *Miswak, Toothbrush, Oral hygiene, Microbiological effect.*

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Introduction

It is well known that removing dental plaques and food debris regularly to maintain oral hygiene is regarded as an essential factor in the prevention of poor oral health, the combined use of a toothbrush and toothpaste is among the most common oral hygiene maintenance methods, followed by mouthwash and dental floss[1].

Miswak, or chewing stick, which was prepared from roots and twigs of different species of plants and was the most effective tool used anciently, has ignored the emergence of modern toothbrushes and interdental cleaners[2]. Easy availability, simple use, cultural and religious factors, and the low cost of this natural product made many developing countries continue using it for tooth cleaning[3,4]. Chewing sticks for tooth cleaning were practiced in the ancient world by Arabs, Babylon, Greeks, and Romans; it was also used in the Middle East, Asia, Africa, and the US, thousands of years ago[5].

Later, after using for many years as a tooth cleaning tool, new effects of Miswak on oral hygiene were discovered. Natural ingredients like ascorbic acid, tri-methylamine, chloride, fluoride, silica, resins, and salvadorine, made Miswak an important tool for oral health. These natural contents have proved potency to heal the inflamed and bleeding gums, produce a stimulatory effect on the gingiva, remove tartar, and other stains from the teeth, re-mineralize dental hard tissue whitens teeth, provide enamel barrier, and increase salivary flow. In addition, researchers revealed that Miswak has anti-septic, astringent, and bactericidal properties that help reduces plaque formation, provides anti-carious effects, eliminates bad odor, improves the sense of taste, and cure many systemic diseases through the presence of volatile oils, tannic acid, sulfur and sterols within its ingredients[6].

Miswak, an Arabic word that means tooth-cleaning stick, becomes a common name for *Salvadora persica* due to its widespread association with the practice[8].

Sticks from these plants are usually chewed or tapered on one end until they became frayed into a brush-like form, which is then used to clean the teeth, similar to a toothbrush[9]. Two-finger and 5-finger grip techniques are shown in Figure 1, a –b[10,11].

In the year 2000, an international consensus report on oral hygiene concluded that miswak could be an excellent oral hygiene tool. Further research should be done to test its efficacy against oral pathogens[12]. The discovery and apprehension of the alternate oral practices, particular reference to these chewing sticks, was recommended in 1987 by The World Health Organization and is still being allowed to support these as an effective tool for oral hygiene[13,14].

Gram-negative bacteria such as Tannerella forsythia (Bacteroides forsythias), Porphyromonas gingivalis, and Treponema denticola are significantly higher levels in patients with chronic and aggressive periodontitis and therefore often called the complex red bacteria. These Gram-negative bacteria, along with Aggregibacter (Actinobacillus) actinomycetemcomitans, colonize the gingival pockets; they are strong inducers of inflammation and tissue destruction and with a highly developed capacity to evade host immune responses and resist antimicrobial treatment[15,16].

The study aims to compare Miswak and manual toothbrush users, estimating oral manifestations, oral hygiene, OHI-S, gingival index (GI) and DMFT index, and microbiological and mechanical effect on the oral cavity.

Figure 1: (A) Miswak, (B) Manipulation of miswak for oral hygiene maintenance; palm grip for holding[10,11].

All over the world, about182 species of plants are used as chewing sticks. *Salvadora persica* is the most commonly used plant for this practice[7]. *Salvadora persica* plant is commonly known as the miswak tree.

Subjects and methods

In this prospective observational study, the clinical examination of each patient was done intra-orally to detect oral manifestation by using a prepared case sheet in a private dental clinic of the researcher. The

bacteriological part of the study was carried out at the microbiological laboratory in Kirkuk General Health Laboratory/Kirkuk. This study was conducted from May 2015 till April 2016.

The study sample consists of 60 males, their age range between 25-65 years. They were divided into Group A: Miswak users (test group) and group B: toothbrush users (control group), each group consisting of 30 persons. Group A is using a chewing stick (Salvadora persica) as (test group): the participants of this group should be those using their Miswak (i.e., on their respective allocations) for five years and more. Group B uses nylon manual toothbrushes as (control group); the participants of this group should do brushing three times daily (after breakfast, after lunch, and before going to bed) for five years and more.

Ethical considerations: The study was approved by the medical ethical committee, University of Sulaimani. Informed patient consent will be taken from all the participants in the study.

Inclusion criteria: Healthy individuals with ≥18 teeth; in both groups of Miswak or toothbrush users; who used their Miswak or toothbrush for not more than five years.

Exclusion criteria: include any individuals who are smokers, used antibiotic in the last three months, used anti-septic mouthwash, with a history of systemic diseases, with oral manifestations of systemic diseases or medications and with fully crowned teeth or dental prosthesis and orthodontically bonded teeth.

Materials and equipment

Mirror and explorer probe.World Health Organization (WHO) probe[16], which has a 0.5-mm ball at the tip and millimeters markings at 3.5, 5.5, and 11.5 mm and color-coding from 3.5 to 5.5 mm.

The clinical examination included Simplified Oral Hygiene Index (OHI-S) according to (Greene and Vermilion, 1964), and gingival index according to (Loë H and Silness J, 1963), DMFT index according to (Klein et al. 1938)[17,18,19].

Selection of tooth surfaces: The six surfaces examined for the OHI-S are selected from four posterior and two anterior teeth, Figure: 2, a –b.

- Posterior dentition portion; the first fully erupted tooth distal to the second bicuspid (15) was examined. Mostly the first molar (16) but sometimes the second (17) or third molar (18). The inspection of the buccal surfaces of the selected upper molars and the lingual surfaces of the selected lower molars was performed.
- Anterior mouth, the scoring of the upper right (11) labial surfaces, and the lower-left central incisors (31) were made. In the absence of either of these anterior teeth, the central incisor (21 or 41 respectively) on the opposite side of the midline is substituted.

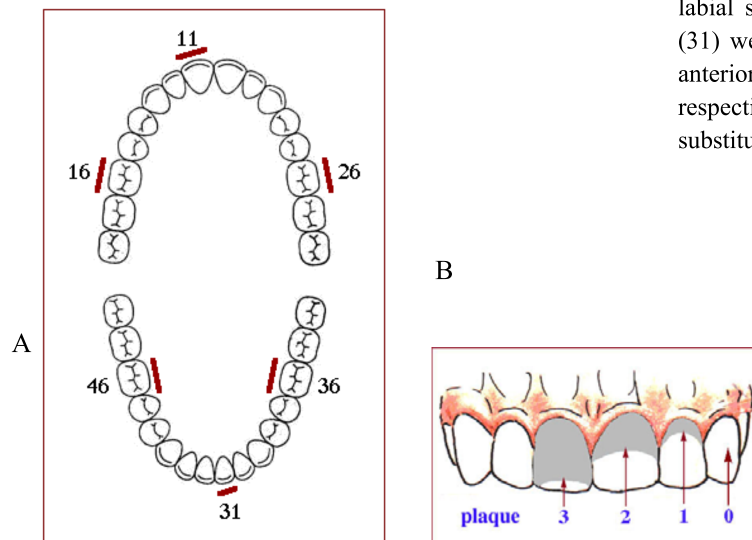

Figure (2, A-B). Selection of tooth surfaces: The six surfaces examined for the OHI-S are selected from four posterior and two anterior teeth. OHI-S)[17,18].

Saliva samples:

The participants were asked not to eat or drink for one hour before saliva collection. Each participant was asked to intake 5 ml distal water and vortex mix it inside the mouth for 1 minute, then recollecting it within sterile plastic cups by spitting method. Saliva samples were collected between 8 and 10 a.m. and were immediately stored at 20C, each cup labeled and sent directly for microbiological examination at Kirkuk General Health Laboratories/Kirkuk.

Isolation of Microorganisms

A saliva sample was processed microbiologically for quantitative study following accepted laboratory methods. Using the microbiological loop, 0.01 ml saliva was spreading in the culture media: Under aerobic environment and at 35 ± two °C, the samples were incubated. After 24 hours of culture, the reading was done, and the first reading was recorded, and a final one was recorded after 48 hours as a second reading. Following microbiological media were used for isolation of different pathogenic and nonpathogenic bacteria from the samples; MacConkey agar (MAC), Mannitol salt agar (MSA), Nutrient agar (NA), Chocolate agar (CHOC), blood agar, and Sabouraud Dextrose Agar (SDA).

Identification of bacterial isolates

Both primary and confirmative identification of bacteria was performed. Several biochemical tests were examined to identify the bacteria of interest. The isolated bacteria are further processed through one or a few of these procedures to identify the bacteria, staining the isolated bacteria, and biochemical testing.

Statistical analysis

The data were analyzed using the Statistical Package for Social Sciences (SPSS, version 19). The oral findings between groups A and B, miswak, and toothbrush users were compared using the Student T-test. The descriptive variables; different microorganism findings were shown as numbers and percentages. The comparisons between them were performed using the Chi-square test, with the P-value of less than 0.05 as a significant level.

Results

The total number of the studied sample was 60, which were composed of two groups. Group A Miswak users (n = 30); and Group B toothbrush users (n = 30) served as a control group. The mean age (±SD) of the miswak group was (37.4 ± 10.9), while for the toothbrush group was (33.0 ± 4.40). (p = 0.04).

Mean oral hygiene index-simplified (OHI-S), gingival index (GI), and the mean DMFT (Decay-Missing-Filling Teeth) for the miswak users, the toothbrush users, there was a statistically significant difference between the two groups regarding these three oral findings with (p = 0.001), as shown in Table 1.

In comparison between both study groups regarding gum recession, there was a highly significant difference, with (P = 0.01) and a significant difference regarding abrasion (P = 0.001, as shown in Table 2.

Regarding the microorganisms among both groups (A and B), there was a significant difference regarding *E. coli* and *Candida albicans* and a significant difference regarding *Klebsiella and Diphtheroids.* In contrast, no significant differences were observed regarding *S.pyogenes, Lactobacillus, and Staph. Aureus* in their saliva samples, as shown in Table 3 and Figure 4.

Discussion

The present study revealed a statistically significant difference in oral hygiene index by measuring both plaque and calculus among either Miswak or toothbrush users (P=0.001). This demonstrates that miswak users could not control their oral hygiene as effective as those who used a toothbrush. This agrees with the result conducted by Norton and Addy 1989[20] and disagrees with results conducted by Raghavendra et al.[21].

On the other hand, the present study results demonstrate a significant difference between miswak and toothbrush users regarding gingivitis. A toothbrush was more effective in the reduction of gingivitis compared to miswak (p=0.001). This finding was in agreement with several previous studies[22,23].

As shown in (Table 2) and (Table 3), the results of this study suggest that chewing stick(Miswak), even though they contain antimicrobial compounds, were found by El-said et al.[24] and Rotini and Mosadomi[25], offered no advantage to oral hygiene and gingival health

Table 1: Oral findings; OHI-GI-and DMF of miswak and toothbrush groups.

Measure	Group A No.=30 Mean (SD)	Group B No.=30 Mean (SD)	p-value
O.H.I	1.92 (0.50)	0.81 (0.27)	0.001
G.I	1.79 (0.34)	0.72 (0.19)	0.001
DMFT	11.1(6.67)	2.67 (2.80)	0.001

Table 2: Comparison between the study groups regarding gum recession and abrasion.

Groups	**Gum recession****		**Abrasion***		**Total**
	Yes	**No**	**Yes**	**No**	
A	22	8	13	17	30
	73.3%	26.7%	43.3%	56.7%	100%
B	6	24	4	26	30
	20%	80%	13.3%	86.7%	100%
Total	28	32	17	43	60
	46.7%	53.3%	28.3%	71.7%	100%

Table 3**:** The number and percentage of different microorganisms among miswak (group A) and toothbrush (group B) users.

Microorganism	**Group A No.=30 Number (%)**	**Group B No.=30 Number (%)**	**p-value**
S.pyogenes	22 (73.3)	18 (60)	0.27
Klebsiella	12 (40%)	5(16.7)	0.04*
E.coli	9(30)	1 (30.3)	0.006**
Candida albicans	8 (26.7)	0 (0)	0.002**
Diphtheroids	30 (100)	25(83.3)	0.02*
Lactobacillus	7 (23.3)	10(33.3)	0.39
Staph.aureus	17 (56.7)	22 (73.3)	0.17

*Significant, **highly significant

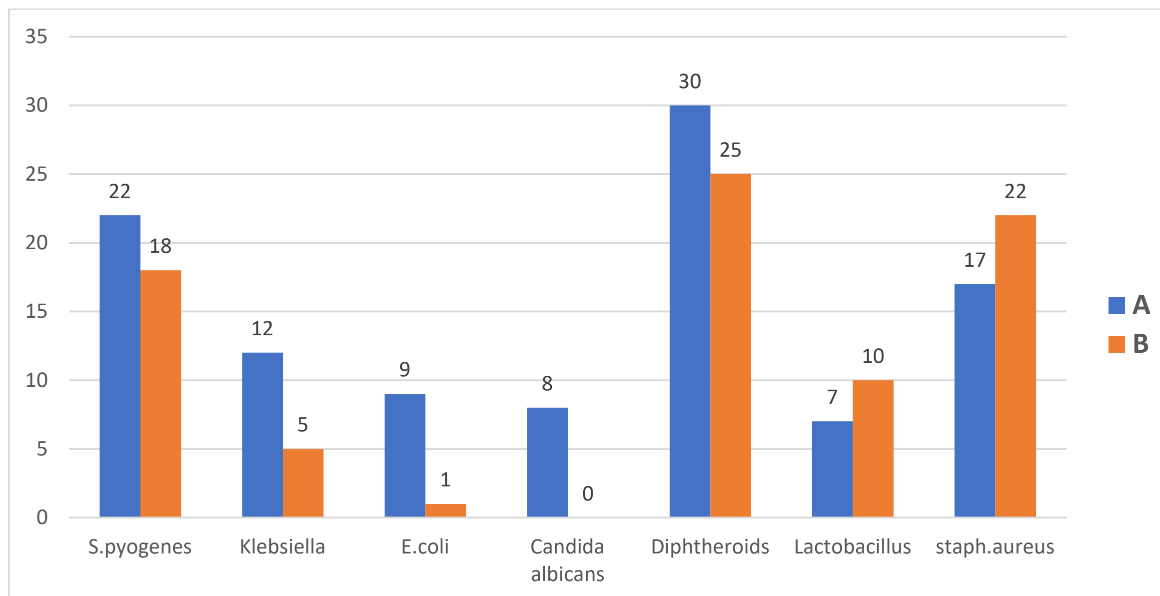

Figure 4**:** Different microorganisms isolated from saliva samples of both study groups (group A and group B).

over toothbrush with toothpaste. Overall, plaque scores were significantly high in individuals who used miswak compared with those who used toothbrushes. Moreover, the increased plaque was sufficiently large to be reflected by a significant increase in gingival bleeding in the miswak users.

There are two possible explanations for the observed differences: first, miswak and toothbrushes have different designs but similar functions. In the miswak use, the surfaces of the teeth that could be reached easily are the facial surface rather than the lingual and interdental spaces, due to the location of the bristles along the length of its handle, unlike the conventional toothbrush(26). Thus, easy access to the whole teeth surfaces could be impossible. On the other hand, the angulations of toothbrushes allow the user to reach distal tooth surfaces, particularly on the posterior teeth, with greater ease. Most of the participants in (group A) in this study were employed and reported having little time for tooth cleaning with miswak properly. There is a need to soften and splay the end of miswak before cleaning can begin; time is required with the stick than with a toothbrush.

The present study showed that the mean DMFT is higher in group A (miswak user) than that in group B (toothbrush users) and the difference was statistically significant (P=0.001). This difference may be due to many health and social factors (oral hygiene, carbohydrate consumption, fluoride application, dental education, socioeconomic status, preventive programs, and social habits). The results showed a higher caries experience in the miswak users, and this disagrees with the results of previous studies by Halawany HS(27); and the authors concluded that caries in adults was mostly present in older persons and usually involved the maxillary and mandibular second and first molar which are difficult to reach for cleaning with miswak(28).On the other hand, the result showed the lower caries experience in subjects who use a modern toothbrush, and this is agreed with the study by Nyvad(29), who concluded that daily tooth brushing with fluoride toothpaste is believed to be the primary reason for the caries decline that has been observed since 1970. Studies have shown that the frequency of tooth brushing had a significant association with caries prevalence(30).

Good oral hygiene and tooth brushing twice a day at an early age were factors associated with good dental health. As shown in (Table 2), gingival recession in the study was increased significantly in miswak users compared to toothbrush users (p=0.01). This was in agreement with Eid et al.(26), who noticed that the severity of the gingival recession was significantly more pronounced in the miswak users than it was in the toothbrush users. Also, the present study results paralleled that of Norton and Addy(19) and who reported that a marked gingival recession frequently accompanies miswak. This is maybe because gingival recession induced by miswak is partly due to improper technique or trauma from hard fibers of miswak on the gingival tissues proposed by Akhter and Ajmal(31).

This study showed that tooth wear (abrasion) significantly increased in miswak users in comparison to toothbrush users (p=0.001) as shown in (Table 2), in which (43.3%) of subjects in group A were had tooth

abrasion, while in group B it was only 13.3%, and this agrees with the result conducted by Hollist[10], this difference may be because excessive scrubbing of miswak on the anterior teeth by permanent miswak users compromised the aesthetic zone and resulted in severe attrition of the anterior teeth as proposed by Khoory[32], claimed miswak as one of the etiology of gingival recession.

The present study assessed and compared the levels of different microorganisms in the salivary sample in a sample of people in Kirkuk city who were habitual miswak and toothbrush users and various microorganisms isolated. These microorganisms were associated with various diseases such as Lactobacilli, leading to the progression of dental caries. Candida results in candidiasis, *Klebsiella* associated with pyogenic infection, pneumonia, septicemia, and diarrhea, *E.coli* causes septicemia, diarrhea, and urinary tract infection, and *Strep. pyogenes* cause pharyngitis and cellulitis Karibasappa *et al.*[33].

After taking a saliva sample from both miswak and toothbrush users and isolating seven types of specific microorganisms, the subjects showed a statistically significant reduction of *Klebsiella, Candida, E. coli,* and *Diphtheroids*. The remaining three bacterial species showed no significant changes after the use of either of the two devices. It has been demonstrated that extracts of miswak have inhibitory effects on the growth of several oral microorganisms, such as *Streptococcus mutans, Streptococcus faecalis, P. gingivalis, P. intermedia, T. denticola, F. nucleatum, E. corrodens, and C. rectus* Almas & Al-Bagieh [34].

Darout et al.[35] in 2002, compared the salivary levels of 25 oral bacteria between habitual miswak and toothbrush users among adult Sudanese subjects, and their findings suggested that the miswak might have selective inhibitory effects on the level of certain bacteria in saliva, particularly several oral streptococci species. In that study, *A. actinomycetemcomitans* and other bacterial species were present in significantly higher numbers in the saliva of miswak users than in the toothbrush users. Furthermore, it is revealed in a recent study that miswak users held significantly higher plaque levels of *S. intermedius, A. actinomycetemcomitans, Veillonella parvula, Actinomyces israelii, and Capnocytophaga gingivalis* and significantly lower *Selenomonas sputigena, Streptococcus salivarius, Actinomyces naeslundii, and S. oralis* than did toothbrush users Darout et al. 2003[36].

Regarding *Candida albicans* which is responsible for multiple infections in the oral cavity, as shown in table (3), there was a significant increase in the level of *candida Albicans* in saliva sample of miswak users than in toothbrush users (P=0.002), and this is in agreement with the previous results found by (Almas and Al-baghi) who concluded that miswak ineffective against *Staph.aureus, S.epidermitis, and C.albicans*[34].

Regarding *Staph. Aureus* in the present study, more than 73% of participants in group A had this type of bacteria in their saliva samples as shown in table (3), and this may be because conducted by Sher H. and his colleague when they found that methanol extract of Salvadora persica (Miswak) demonstrated the least activity against *S.aureus*[37].

Regarding other pathogenic microorganisms (*Klebsiella*, *E. coli*, and *Strep. pyogenes*) were present in significantly higher numbers in saliva sample of miswak than in the toothbrush group, as shown in table 3 and figure 4, and this is in agreement with the results conducted by Bashair and Ohood when they compared bacterial contamination of miswak and toothbrush[38], these differences may be due to that miswak remain inactive in interproximal surfaces, suggesting its limiting action on micro-flora in these surfaces Adnan et al.[39], another reason is maybe due to extensively contamination of miswak with a variety of microorganisms, because most of the participants of miswak users in this study, they carry it with them on their hands or inside their pockets, which is very unhygienic.

Moreover, the antimicrobial agents contained in the miswak offer no advantages over tooth brushing with toothpaste. However, it must be remembered that toothpaste also possesses antimicrobial action, which is demonstrable *in vitro* and *in vivo* Moran et al. [40].

Conclusions

Oral hygiene index-simplified and gingival index was higher among Miswak users than toothbrush users. The mean DMFT, gum recession, and tooth wear (Abrasion) were significantly higher in Miswak users than in toothbrush users.

Pathogenic microorganisms like (*Klebsiella, Candida albicans, E.coli)* were significantly higher in saliva samples of Miswak users than in toothbrush users. At the same time, there were no significant differences

between Miswak and toothbrush users regarding the isolation of *Strep. Pyogenes*. *Diphtheroids* were high among Miswak and toothbrush users with a higher level in toothbrush users than Miswak users.

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References

1. Van Leeuwen MP, Rosema NA, Versteeg PA, Slot DE, Hennequin-Hoenderdos NL, Van der Weijden GA. Effectiveness of various interventions on maintenance of gingival health during 1 year–a randomized clinical trial. Int J Dent Hyg. 2017;15(4):e16-27.
2. Hyson JM., Jr.History of the toothbrush. J Hist Dent. 2003;51(2):73-80.
3. F. Niazi, M. Naseem, Z. Khurshid, M.S. Zafar, K. AlmasRole of Salvadora persica chewing stick (miswak): A natural toothbrush for holistic oral health. Eur. J. Dent. 2016;10(2):301-08.
4. Owens J, Sami W. The role of the Qur'an and Sunnah in oral health. J Relig Health. 2016;55(6):1954-67.
5. Gurudath G, Vijayakumar KV, Arun R. Oral hygiene practices: ancient historical review. J Orofac Res. 2012:225-7.
6. Dahiya P, Kamal R, Luthra RP, Mishra R, Saini G. Miswak: A periodontist's perspective. J Ayurveda Integr Med. 2012;3(4):184.
7. Qureshi AA, Qureshi AA, Dohipoide A, Jamadar NN. Effects of miswak—Salvadora persica on oral health. Al Ameen J Med Sci. 2016;9(4):215-8.
8. Haque MM, Alsareii SA. A review of the therapeutic effects of using miswak (Salvadora Persica) on oral health. Saudi Med J. 2015;36(5):530.
9. Ahmad H, Ahamed N. Therapeutic properties of meswak chewing sticks: A review. Afr J Biotechnol. 2012;11(83):14850-7.
10. Hollist NO. The technique and use of chewing stick. Odontostomatol Trop. 1981;4(3):171-4.
11. Almas K, Al-Zeid Z. The immediate antimicrobial effect of a toothbrush and miswak on cariogenic bacteria: a clinical study. J Contemp Dent Pract. 2004;5(1):105-14.
12. Al-Bayati FA, Sulaiman KD. In vitro antimicrobial activity of Salvadora persica L. extracts against some isolated oral pathogens in Iraq. Turk J Biol. 2008;32(1):57-62.
13. Ababneh H. The effect of the extract of the miswak (chewing sticks) used in Jordan and the Middle East on oral bacteria. Int Dent J. 1995;45(3):218–22.
14. Khalessi AM, Pack AR, Thomson WM, Tompkins GR. Extracts of Salvadora persica. Int Dent J. 2004;54(5):279–83.
15. Ezzo PJ, Cutler CW. Microorganisms as risk indicators for periodontal disease. Periodontol. 2003;32(1):24–35.
16. Marsh PD. Controlling the oral biofilm with antimicrobials. J Dent. 2010;38(1):11-15.
17. Green JG and Vermillion JR. The Simplified Oral Hygiene Index. J Am Dent Assoc. 1964,68: 7-13.
18. Löe H, Silness J. Periodontal disease in pregnancy I. Prevalence and severity. Acta odontol Scand. 1963;21(6):533-51.
19. Klein H, Palmer CE. Studies on dental caries: V. Familial resemblance in the caries experience of siblings. Public Health Reports (1896-1970). 1938;53(31):1353-64.
20. Norton M.R and Addy M. Chewing sticks versus toothbrushes in West Africa. Clin Prev Dent. 1989;11(3):11-3.
21. Shetty RM, Shetty S, Sachin BM, Amirisetty R, Agrawal A. Comparative study to assess the effect of chewing stick and toothbrush on oral hygiene and periodontal status among Indian population. Int J Public Health Dent. 2010;1(1):6-12.
22. Gazi M, Saini T, Ashri N, A. L. Miswak chewing stick versus conventional toothbrush as an oral hygiene aid. Clin Prev Dent. 1990;12(4):19-23.
23. Al-Otaibi M, Al-Harthy M, Söder B, Gustafsson A, Angmar-Månsson B. Comparative effect of chewing sticks and toothbrushing on plaque removal and gingival health. Oral Health Prev Dent. 2003;1(4):301-7.
24. El-Said F, Fadulu SO, Kuye JO, Sofowora EA. Native cures in Nigeria. The antimicrobial properties of the buffered extracts of chewing sticks. Lloydia. 1971;34:172-4.
25. Rotimi VO, Mosadomi HA. The effect of crude extracts of nine African chewing sticks on oral anaerobes. J Med Microbiol. 1987;23(1):55-60.
26. Eid MA, Selim HA, al-Shammery AR. Relationship between chewing sticks (Miswak) and periodontal health. Part 1. Review of the literature and profile of the subjects. Quintessence Int. 1990;21(11):913-7.
27. Halawany HS. A review on Miswak (Salvadora persica) and its effect on various aspects of oral health. Saudi Dent J. 2012;24(2):63-9.
28. Ismail A.Darout, Nils skaug, . Comparative oral health status of an adult Sudanese population using

miswak or toothbrush regularly. Saudi Dent J. 2004;16(1):411-20.
29. Kidd EA, Fejerskov O. Dental caries: The disease and its clinical management. Blackwell Munksgaard; 2003.
30. Stecksen-Blicks C, Sunnegardh, K, and Borssen, E. Caries experience and background factors in 4-year-old children: time trends 1967-2002. Caries Res. 2004;38(2):149-55.
31. Akhtar MS, M. A. Significance of chewing sticks (miswaks) in oral hygiene from a pharmacological viewpoint. J Pak Med Assoc. 1981;31(4):89-95.
32. Khoory T. The use of chewing sticks in preventive oral hygiene. Clin Prev Dent. 1983;5(4):11-4.
33. Karibasappa GN, Nagesh L, Sujatha BK. Assessment of microbial contamination of toothbrush head: An in vitro study. Indian J Dent Res. 2011;22(1):2-5.
34. Almas, K. & Al-Bagieh, N. H. The antimicrobial effects of bark and pulp extracts of miswak, Salvadora persica. Biomed Lett. 1999;60(235):71–5.
35. Darout, I. A., Albandar, J. M., Skaug, N. and Ali, R. W. Salivary microbiota levels in relation to periodontal status, experience of caries and miswak use in Sudanese adults. J Clin Periodontol. 2002;29(5):411-20.
36. Darout, I.A., Skaug, N., Albandar, J.M. Subgingival micro- biota levels and their associations with periodontal status at the sampled sites in an adult's Sudanese population using miswak or toothbrush regularly. Acta Odontol Scand. 2003;61(2):115-22.
37. Sher H. Al-Yemeni MN, Wijaya L. Ethnobotanical and antibacterial potential of Salvadora persica: A well-known medicinal plant in Arab and Urani system of medicine. J Med Plant Res. 2011;5(7):1224-9.
38. Bashair TA, Ohood FM. Bacterial contamination of toothbrushes and miswak. Indian J Dent Res. 2012;22(1):3-6.
39. Adnan S, Salih M.A., Pranee L, Farzeen Tanwir. Efficacy of miswak on oral pathogens. Dent Res J. 2013;10(3):314-20.
40. Moran J, Addy M, Wade W. Determination of minimum inhibitory concentrations of commercial toothpastes using an agar dilution method. J Den. 1988;16(1):27-31.