Original Article

Paraclinical Effects of Miswak Extract on Dental Plaque

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ABSTRACT

Introduction: The Persian toothbrush tree or Miswak (*Salvadora Persica L.*) has been used as a brushing stick for more than 1,300 years. Pharmacological studies indicated antibacterial and anti-inflammatory activities of Miswak extract. The present study was performed to determine antibacterial effects of Miswak extract.

Material and Methods: The present experimental research involved three *in vitro* studies including: 1) *in vitro* testing of the effect of Miswak extract on selected bacteria; 2) comparing the paraclinical effects of Iranian toothpaste containing Miswak extract and placebo toothpaste on dental plaque; and 3) comparing the antibacterial effect of Iranian toothpaste with Swiss toothpaste (Quail Miswak) on dental plaque. The disc diffusion method was used to test bacterial sensitivity of toothpastes. Data were analyzed by paired t-test and ANOVA.

Results: In the first study, Miswak extract inhibited the growth of some dental plaque bacteria. In the second study, antibacterial effect of the herbal toothpaste was significantly greater than that of the placebo (P =0.002). In the third study, four samples of dental plaque bacteria were used and there was no difference between the antibacterial effects of Swiss and Iranian herbal toothpastes (P =0.66).

Conclusion: Due to antimicrobial effects of Miswak extract, its use in mouth rinses and toothpastes is highly recommended.

Key words: Dental plaque, Plant extract, Salvadora Persica, Toothpaste.

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Introduction

The scientific name of Miswak is "*Salvadora Persica.*" The word Persica is derived from Persian and indicates that Iran is the main growing area of this plant. This plant belongs to the *Salvadoraceae* family, a crowded evergreen shrub that has a soft inclined to a white wood. Since the brushes made of its wood strengthen the gums, it has been called "Miswak tree" in traditional medicine [1]. Chemical compounds such as sodium chloride, calcium oxalate, silica, fluoride, sulfated compounds, vitamin C and tannic acid have been found in this plant [2-4]. Moreover, this plant contains saponin, flavonoid, an alkaloid named Salvadorini, Trimethylamine, an herbal steroid named beta-sitosterol and benzyl isothiocyanate [1,5]. It is claimed that the vitamin C and sitosterol content of this plant have great roles in strengthening the gum capillaries and preventing gum inflammation [2,5,6]. Sulfated compounds and isothiocyanate are known to be responsible for antibacterial effects of the plant, while fluoride and calcium salts are quite effective in preventing dental caries [5,7,8]. Moreover, the silica and calcium salts in the plant act as grinder and detergent [3,9]. Trimethylamine is known to be effective in reducing surface adhesion and also in decreasing plaque accumulation. Tannins, tannic acid and benzyl isothiocyanate, are reported to have antimicrobial effects and help the healing of gum inflammation [3,5,10].

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Leaves, fruits and seeds of this plant have been used in traditional medicine as appetizer, mild laxative, diuretic and anti-fungal medication [1] and people in some Asian and African countries have used it for many years. During recent years, many researchers throughout the world have studied Miswak as a helpful plant in mouth and teeth hygiene [11-14]. The aim of this research was to evaluate the effects of Miswak extract on dental plaque. This study was done for the first time in Iran.

Materials and Methods

In this experimental study, the Agricultural Organization of Kerman Province prepared dried branches of Salvadora Persica taken from the trees along Bandar Abbas/Minab Road in south Iran. After drying and grinding the tips of the branches in the medical laboratory, the extract was prepared using methanol 80% and soxhlet extractor (Bruan, Germany). In the first study, during an *in vitro* evaluation, the antibacterial effect of Miswak extract against *Streptococcus Sanguis*, *Salivarius*, *Eikenella Corrodens* and *Porphyromonas Gingivalis* was studied. These bacteria were prepared in lyophilized form from the Persian Type Culture Collection of the Iranian Research Organization for Science and Technology. The aim of the first stage was to determine the required concentration of the plant for exerting the antimicrobial effects. The lyophilized bacteria were resuscitated in the sterile condition of the laboratory through the following method: first, a scratch was created on the closed glass tube containing bacterium and the tube was thoroughly sterilized by alcohol 70% smeared gas. Then, the tube was broken from the scratch site and 0.3-0.4 ml of tauroglycolate (Himedia, India) was added to the dry content of the tube. After unifying the suspension, it was transferred to the solid culture medium of each bacterium prepared from the Iranian Research Organization for Science and Technology. Culture media were incubated at 37ºC for 48 hours in order to allow bacterial growth. The prepared extract was solved in ethanol 3% and 1/2-1/16 attenuations were prepared. In the next step, 0.5 ml of 1/500 McFarland of each microbial suspension prepared from each bacterium was added to each of the four attenuations. These solutions were incubated and studied for the presence of opacity (opacity was a sign of bacterial growth). In the case of bacterial non-growth, 0.1 ml of the tube content was inoculated to the culture medium. Bacterial growth and colonization in this condition showed the ability of extract in inhibiting bacterial growth, but its inability in killing the bacterium. Then, based on the extract concentration, minimum inhibitory concentration (MIC) for each bacterium was determined.

In the second study, Miswak extract was inserted into a conventional toothpaste compound (Tolidaru, Iran) in order to evaluate the antimicrobial effects of the toothpaste against dental plaque in comparison with the placebo toothpaste produced by Tolidaru Company. The disc diffusion method was used for testing bacterial sensitivity. Twelve students referred to the dental clinic were selected and their bacterial plaque sample was taken from labial surfaces of their teeth using a sterile curette (Hu-Friedy, USA). Plaque samples were transferred to the culture medium. After bacterial growth, a microbial suspension was prepared and transferred to the plates containing culture medium by a sterile swap. Then, sterile paper discs with a diameter of 5 mm (Blank disc, Patan Teb, Iran) were smeared with herbal and placebo toothpastes and were placed on the surface of the plates containing culture medium in equal distances. Because the paper discs were smeared with equal amounts of toothpastes, a pea-size amount of each toothpaste was put on the lamella, the disc was put on the toothpaste and another lamella was put on the first lamella. The two lamellas were pressed on each other for less than 10 seconds, so that all discs were smeared with equal amounts of the toothpastes. Then, the plates were incubated at 35ºC for 16-18 hours. After this period, the area of growth nonexistence around the discs and the diameter of the inhibition zones, which were measured in millimeters, characterized relative bacterial sensitivity to the toothpastes.

In the third study, the effects of two toothpastes containing Miswak extract (one produced in Iran, which had been used in the second study as well, and the other produced in Switzerland named Qulimiswak) on samples of dental plaque bacteria were compared once with each other and once with penicillin. The selected bacteria were *Streptococcus Sobrinus*, *Sanguis*, *Salivarius*, and *Actinomyces viscosus*. The mentioned bacteria were transferred on the culture medium and after their growth and preparation of microbial suspension, like the second stage of the study, bacterial sensitivity to the toothpastes and penicillin (Patan Teb, Iran) was

evaluated using the disc diffusion method. In performing the second and third studies, sterile discs free of any material were used as negative controls in the culture medium. Data were analyzed by paired t-test and ANOVA. P<0.05 was considered statistically significant.

Results

According to the results of the first study, Miswak extract with concentration of 74 mg/ml inhibited the growth of four mentioned bacteria (table 1). In the second study, a significant statistical difference was found between the herbal toothpaste and the placebo in regard to their effects on samples of bacterial plaque (P=0.002). Specifically, the herbal toothpaste was 13.63% more effective than the placebo in plaque control (table 2). In the third study, there was no significant statistical difference between the antibacterial effects of Iranian and Swiss herbal toothpastes (P=0.66), but there was a significant difference between the effects of two toothpastes and that of penicillin (P=0.002) (table 3).

Table 1. Minimum inhibitory concentration (MIC) of herbal extract against microorganisms.

Name and Herbal Extract concentration	Bacteria	Concentration and Bacteria Growth				MIC
		1⁄ 2	1/4	1/8	1/16	
Miswak (Salvadora Persica) 7.4 mg/ml	S. Sanguis	-	-	+	+	1.8
	S. Salivarius	-	+	+	+	3.7
	E. Corrodens	-	+	+	+	3.7
	P. Gingivalis	-	-	+	+	1.8
Witness (Solvent) Ethanol 3%	S. Sanguis	+	+	+	+	0
	S. Salivarius	+	+	+	+	0
	EK. Cordenes	+	+	+	+	0
	P. Gingivalis	+	+	+	+	0

+: Bacterial growth adjacent to special concentration of extract
- : Lack of bacterial growth

Table 2. Comparison of mean diameter of inhibition zone around discs smeared to herbal and placebo toothpastes (dimensions are in millimeters).

Material	Mean	Standard deviation
Smeared to herbal toothpaste	17.75	3.43
Smeared to placebo toothpaste	15.33	3.17
Paired T- test	D.F=11, T= 3.95, P =0.002	

Table 3. Comparison of mean diameter of inhibition zone around discs smeared to Swiss herbal toothpastes, Iranian herbal toothpastes and penicillin (diameters are in millimeters)

Material	Mean	Standard deviation
Smeared to Swiss toothpaste	14.62	4.64
Smeared to Iranian toothpaste	10.75	3.71
Penicillin	32.12	8.98
ANOVA	P = 0.002	

Discussion

Daily use of an efficient anti-plaque compound, especially a formulated form in toothpaste, can be very beneficial in plaque control. Some groups of antimicrobial compounds have been studied thus far. The most important of these compounds are herbal extracts, metallic salts and phenol compounds. Each of these three groups has demonstrated positive results in clinical and laboratory studies. Herbal extracts have received special attention because of being non-chemical and non-synthetic, and they have been long used in traditional medicine [12,15,16]. In an *in vitro* study per-

formed by Patel on the anti-plaque effects of chicory extract, after adding herbal extract to the combination of four different commercial toothpastes, the anti-plaque effects of the mentioned toothpastes in comparison with the same toothpastes without herbal extract were evaluated using bacterial sensitivity tests and discs. Results of this study demonstrated a greater anti-plaque effect in all toothpastes containing herbal extract in comparison to the same toothpastes without extract [7]. The results of the second part of the current comparing herbal and placebo toothpastes, are very similar to the Patel's findings.

In another study, Patel compared the anti-plaque activity of chicory extract with the anti-plaque activity of penicillin, tetracycline, chloramphenicol, and streptomycin using microbial sensitivity tests and discs. In his study, bacteria in plaque samples showed high sensitivity to chloramphenicol and streptomycin, and their sensitivity to chicory extract was between the sensitivity to chloramphenicol and streptomycin [7]. Settembrini and colleagues evaluated the antibacterial sensitivity of six conventional toothpastes against *Streptococci Mutans* and *S. Sanguis* and seperately, *Actinomyces Viscosus*.They used microbial sensitivity evaluation tests with the help of discs. Among the six toothpastes, those containing herbal extract had more preventive effects on *S. Sanguis* and *Actinomyces Viscosus* in comparison with other toothpastes [17]. Likewise in the present research, toothpastes with Miswak extract were the most effective toothpastes on *Actinomyces Viscosus* and *S .Sanguis*.

Almas and co-workers [4] compared the antibacterial effects of Miswak extract with eight commercial mouth rinses. They evaluated the antimicrobial effects on *Pyogenes Faecalis, Mutans Streptococci, Candida Albicans* plus *Aureus* and *Epidermidis staphylococci* by determining the inhibition zones. In their study, none of the solutions was considered a gold standard; they compared the antimicrobial effect of Miswak (mean ± SD) with that of each mouth rinse and the antimicrobial effects of the eight mouth rinses with each other. According to their results, mouth rinses containing chlorhexidine had the greatest antibacterial effects, while mouth rinses containing cetylpyridinium had moderate effect; Miswak extract had a low effect [4]. None of the bacteria used in their study were used in the present study.

Conclusion

The results of three studies of the present research demonstrated that Miswak extract, alone or in combination with toothpaste, can affect the growth of dental plaque bacteria. Therefore, this herbal extract can be used in mouth rinses and toothpastes because of its antibacterial effects and can be beneficial in controlling dental plaque.

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