ABSTRACT: Miswak (Salvadora persica) is one of the oldest known shrubs, being used by millions of people in various parts of the world as an oral hygiene tool. The reason for its wide use is not only its excellent mechanical plaque-removing efficiency but also its broad range of Biological properties. Most importantly, it has good antibacterial and antifungal effects that are responsible for maintaining good oral health including prevention of dental caries and periodontal disease. It also raises the plaque pH and stimulates salivary flow, which play synergistic effect in caries prevention. Besides this, it has protective effect on human leukocytes, inhibitory effect on platelets aggregation induced by thrombin or collagen, increases cell proliferation and promotes wound healing. Some studies have demonstrated its good analgesic, anticonvulsive and sedative effects. Another study also claimed that miswak affects adversely on male and female reproductive system in mice. The purpose of the present article is to provide an overview of various biological effects that will help to understand the importance of this plant and offer suggestions for future research.

KEY WORDS: Biological effects, Miswak, Salvadora persica

INTRODUCTION

‘Miswak’ (or sewak) is a subtropical shrub, native to the Arabian Peninsula, Egypt, and India (Al-Otaibi et al., 2004). It is an Arabic word meaning “tooth-cleaning stick or sticks for rubbing the teeth” and has different names in different cultures: chewing sticks, toothbrush tree, siwak or arak (Almas et al., 2005; Attar, 1979; Darmani et al., 2003; Darout et al., 2000; Dua’a et al., 1996; Hattab, 1997; Khalil, 2006). The scientific name of miswak is Salvadora persica, a member of the Salvadoraeeae family (Darmani et al., 2006). Historically, it is one of the oldest known oral hygiene tools and nowadays, is being used by millions of people in Africa, South America, the Middle East, and Asia (Noui et al., 2010; Sofrata et al., 2008). Miswak stick is prepared from its roots, twigs and stem (Al-Otaibi et al., 2004). When properly prepared and rubbed against the teeth, its fibers stand out like the bristles of a modern toothbrush (Al-samah DA, 1996). It's easy accessibility and the popularity has made it an efficient and inexpensive tool for oral hygiene in different communities (al-Bagieh et al., 1994; Darmani et al., 2006).

The influence of Islam on the spread and use of chewing sticks in different parts of the world is significant (Al-Mohaya et al., 2002). Among Muslims, miswak is usually used at least 5 times daily that is before every prayer. Muslims are still expected to have good oral hygiene before starting their prayers to detoxify and strengthen the weakened gums (Al-Mohaya et al., 2002; Al-samah DA, 1996; Khalil, 2006).

Miswak can provide an excellent oral hygiene due to the mechanical plaque-removing properties of the soft wood fibers and the therapeutic action of its chemical constituents (Al-samah DA, 1996). Several studies have reported the therapeutic effects of the chemical constituents on cariogenic bacteria and periodontal pathogens (Al lafi and Ababneh, 1995; Sote and Wilson, 1995), and also inhibitory action on dental plaque formation (Almas et al., 2005). A single-blind cross-over clinical study on a sample of Saudi Arabian population showed that the miswak is more effective than conventional tooth brushing for reducing plaque and gingivitis, when used with the professional instruction of the proper use of miswak and toothbrush (Al-Otaibi et al., 2004). It has been scientifically demonstrated to be very useful in the prevention of tooth decay and periodontal diseases (Al-Mohaya et al., 2002; Nouni et al., 2010). Baghdady and Ghose reported low caries prevalence among Sudanese schoolchildren, which they attributed to the use of miswak (Baghdady and Ghose, 1979). Recently, Darout et al. indicated that the periodontal status of miswak users in a Sudanese population was similar to that of toothbrush users (Darout et al., 2002). The World Health Organization has recommended and encouraged the use of chewing sticks as an effective tool for oral hygiene in areas where such use is customary (WHO, 1987). It also plays a role in the promotion of oral hygiene, and evaluation...
of the effectiveness of chewing stick requires further research according to the Consensus Statement on Oral Hygiene (2000) (Al-Mohaya et al., 2002; Al-Otaibi et al., 2004). Despite the extensive use of miswak and growing interest in the herbal oral care products, this plant has not received much attention and has not been intensively studied. The purpose of the present article is to provide an overview of various biological effects that will help to understand the importance of miswak (*Salvadora persica*) and offer suggestions for future research.

**BIOACTIVE COMPONENTS OF MISWAK**

A variety of natural components have been identified in *S. persica* extracts, including trimethylamine, salvadoreine, chlorides, fluoride, silica, sulphur, vitamin C, tannins, saponins, flavonoids and sterols (Table 1). Gas chromatography–mass spectrometry analyses of the oil extract of miswak revealed that it also consists of benzylisothiocyanate (BIT), limonene, alpha-pinene and flavonoids (Bader et al., 2002; Sofrata et al., 2008). Some of these beneficial substances are shown to be accountable for the oral health effects of *S. persica* (Noumi et al., 2010). However, there is limited information on the active contributing compounds for the oral health benefits of *S. persica*. The high amount of NaCl, KCl, trimethylamine, salvadourea and salvadoreine in miswak might be responsible for the antibacterial, antiphlogistic and gum-stimulating effects (Hattab, 1997; Monks et al., 1990; Darmani, et al., 2006). Darout et al. hypothesized that thiocyanate from miswak elevates the level of salivary thiocyanate and exerts antimicrobial activity. This could enhance the efficacy of the salivary hydrogen peroxide–peroxidase-thiocyanate system, which is an antimicrobial (Darmani et al., 2006; Darmani et al., 2000; Tenovuo et al., 1981).

**BIOLOGICAL EFFECTS OF MISWAK**

**Anti bacterial effect**

Several in vitro and in vivo studies have been published to demonstrate the antibacterial effect of miswak extract. Different studies used different methods of extraction like aqueous, methanol, ethyl acetate, acetone extract of dry, fresh or crude miswak stems. These studies show the different extracts of miswak have significant inhibitory effects on the growth of several oral microorganisms, such as *Streptococcus mutans*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus mitis*, *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *T. denticola*, *F. nucleatum*, *E. corrodens* and *C. rectus* and *anaerobic Streptococcus* (Al-Otaibi et al., 2004; Al laifi and Ababneh, 2006; Mabrouk et al., 2005; Rajabalian, 2009).

**TABLE 1. Natural components of miswak and their biological effects**

<table>
<thead>
<tr>
<th>Miswak Components</th>
<th>Biological effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride</td>
<td>Antiphlogistic, antifungal, antibacterial, gum stimulating effects removes calculus and extrinsic stains (Darmani et al., 2006; Noumi et al., 2010)</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>Antiphlogistic, antifungal, antibacterial, gum stimulating effects, removes calculus and extrinsic stains (Darmani et al., 2006; Noumi et al., 2010)</td>
</tr>
<tr>
<td>Sulphur-containing organic substances salvadourea,</td>
<td>Antibacterial, antifungal, antiphlogistic and gum-stimulating effects (Darmani et al., 2006; Noumi et al., 2010)</td>
</tr>
<tr>
<td>Oleic acids</td>
<td>Antifungal, protects DNA against reactive oxygen species, antibacterial activity (De Lima et al., 2008; Dilkka et al., 2000; Noumi et al., 2010; Zheng et al., 2005)</td>
</tr>
<tr>
<td>Linoleic acids</td>
<td>Antifungal, antibacterial activity (Dilkka et al., 2000; Noumi et al., 2010; Zheng et al., 2005)</td>
</tr>
<tr>
<td>Trimethylamine</td>
<td>Antiphlogistic, antibacterial and gum-stimulating effects (Darmani et al., 2006)</td>
</tr>
<tr>
<td>Thiocyanate, benzylisothiocyanate and nitrate</td>
<td>Antibacterial, antifungal, antiviral activities and anti-cariogenic properties (Al-Otaibi et al., 2004; Almas et al., 2005; Darmani et al., 2003; A. H. Sofrata et al., 2008; Tubaishat et al., 2005)</td>
</tr>
<tr>
<td>Silica</td>
<td>Abrasive to remove plaque and stains (Darmani et al., 2003)</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Wound healing and tissue repair, anti scorbatic activity, curing spongy and bleeding gums (Darmani et al., 2003; Dilmak, 2006)</td>
</tr>
<tr>
<td>Resins</td>
<td>Protective action by forming a layer over enamel surface after using miswak (Darmani et al., 2003)</td>
</tr>
<tr>
<td>Tannins</td>
<td>Astringent, saliva stimulator (Darmani et al., 2003)</td>
</tr>
<tr>
<td>Saponins</td>
<td>Antibacterial and antifungal activity (Ahmed et al., 2008; Coleman et al., 2010; Darmani et al., 2003)</td>
</tr>
<tr>
<td>N-benzyl-2-phenylacetamide</td>
<td>Antimicrobial activity (Khalil, 2006)</td>
</tr>
<tr>
<td>Lignans</td>
<td>Antimicrobial activity (Khalil, 2006)</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Antibacterial, antifungal, antiviral and cytotoxic activity (Khalil, 2006; Rajabalian et al., 2009; Sofrata et al., 2008)</td>
</tr>
<tr>
<td>Fluoride</td>
<td>Anticariogenic activity and tooth remineralisation (Tubaishat et al., 2005)</td>
</tr>
<tr>
<td>Essential oils</td>
<td>Antibacterial effects and saliva stimulator (Tubaishat et al., 2005)</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>Dentifrice (Tubaishat et al., 2005)</td>
</tr>
<tr>
<td>Alkaloids Nitrogenous containing organic substances salvadoreine</td>
<td>Antifungal, antibacterial, gum-stimulating effects and cytotoxic activity (Darmani et al., 2006; Noumi et al., 2010; Rajabalian et al., 2009)</td>
</tr>
<tr>
<td>Calcium</td>
<td>Inhibits enamel demineralization and promotes tooth remineralisation (Tubaishat et al., 2005)</td>
</tr>
</tbody>
</table>
Aqueous extract of miswak show concentration dependent antimicrobial activity against Strep. faecalis (microbial inhibition zone 7 mm), Staph. Mutans (microbial inhibition zone 3 mm) (Almas et al., 2005), Streptococcus mitis and anaerobic Streptococcus (microbial inhibition zone range from 2 mm to 3 mm) (Tuabashat et al., 2005). Almas et al. demonstrated varied bacterial growth inhibition by aqueous and methanol extracts of miswak (Almas K et al., 1997). Some studies have reported that miswak extracts are effective against Streptococcus mutans (Salehi and Momeni, 2006) and S. faecalis, even when low extract concentrations are used (Almas et al., 1997; Noumi et al., 2010).

N-benzyl-2-phenylacetamide, together with other constituents of S. persica such as lignans and flavonoids, show antimicrobial activity against Escherichia coli (Saleem et al., 2005). At a concentration of 87μg/mL, the extracted N-benzyl-2-phenylacetamide from the miswak has an activity equivalent to 20μg/mL of gentamicin (Khalil, 2006). Sofarata et al. suggested that the studies done using miswak extract probably do not reflect the real antibacterial activity of miswak. Some active compounds may not be extracted or deactivated during preparation of the crude extraction process. He used suspended and embedded pieces of miswak to find the inhibitory effects of volatile oils of miswak on bacterial growth. Miswak stick pieces have strong antibacterial effect against most of the bacterial species than the aqueous miswak extract. It shows strong effect on Gram-negative bacteria P. gingivalis, A. actinomycetemcomitans, and H. influenzae less effect on Gram-positive bacteria S. mutans, and much less effect on L. acidophilus. Both suspended and embedded miswak show very strong dose-response linear correlation inhibitory effect on P. gingivalis (microbial of inhibition zone more than 14-cm) (Sofrata et al., 2008). A 0.14-g suspended miswak (microbial inhibition zone 13 cm) exhibited significantly greater inhibition on A. actinomycetemcomitans and H. influenzae than the miswak embedded in agar (inhibition zone 10.9 cm) (Al-Otaibi et al., 2004). H. influenzae shows the same pattern as A. actinomycetemcomitans with inhibition zone 12.0 cm and 9.3 cm in suspended and embedded miswak respectively. However, the 0.14-g suspended miswak was less effective on S. mutans than the corresponding embedded miswak (inhibition zones of 3.4 cm) and it had no inhibitory effect on L. Acidophilus (Sofrata et al., 2008). Antibacterial effect remains unchanged with miswak suspended 3 mm above the inoculated agar plates, suggesting the presence of volatile active antibacterial compounds (Sofrata et al., 2008).

A clinical study on Sudanese demonstrated that miswak users have significantly lower numbers of cariogenic bacteria in their saliva while the matched toothbrush users had lower salivary levels of periodontic pathogens (Darmani et al., 2006; Darout et al., 2000). Another clinical study has shown significantly greater reduction in S. mutans with miswak use as compared to toothbrushing, whereas no significant difference is observed in reduction of lactobacilli (Almas and Al-Zeid, 2004; Sofrata et al., 2007). The use of miswak reduces A. actinomycetemcomitans significantly more than the ordinary toothbrush. Whole genomic DNA 1050 probes and the CKB method showed that the subgingival plaque samples of the subjects after the miswak use had significantly lower levels of A. actinomycetemcomitans. (Al-Otaibi et al., 2004).

Miswak shows effective antibacterial activity against wide range of bacteria. Compounds responsible for this effect may be sodium chloride, potassium chloride, thiocyanate, saponins, (Hattab, 1997; Monks et al., 1990; Darmani, et al., 2006) and different types of oils. Despite this, there is lack of evidence that which specific miswak compounds is active against which specific bacteria and its mechanism of action. More standardised research also need to be done for other periodontal and cariogenic pathogens.

**Antifungal effect**

*S. persica* also has the potent antifungal effect on different fungal species. Various in vitro studies screened methanol, ethyl acetate, and diluted acetone extracts of dry and fresh *S. persica* stems activity against some Candida species. Miswak inhibits growth and acid production of Candida albicans (Sofrata et al., 2007). Diluted acetone extract (300 mg/ml concentration) of the dry *S. persica* has antifungal activity against oral C. albicans, C. glabrata, and C. parapsilosis strains with the zone of inhibition range: 10.33–15 mm. Whereas, methanol and ethyl acetate extract of dry *S. persica* has lower antifungal activity for oral *C. albicans* with zone of inhibition: 8 mm, when compared against Amphotericin B. Moreover, diluted acetone extract of dry *S. persica* has the highest antifungal activity against *C. albicans*. However, some of the strains like *P. jadinii* CECT 1060, *C. atlantica* CECT 11860, *C. fimata* CECT 11957, and *C. maritima* CECT 1435 are resistant to both dry and fresh *S. persica* stem extracts (Noumi et al., 2010). 15% concentration aqueous extracts of miswak also reduces the growth of *C. albicans* for up to 48 hours (Al Bagieh and Almas, 1997). In contrast, fresh *S. persica* extracts showed no significant antifungal activity against any Candida strains (Noumi et al., 2010).

A clinical study of the prevalence of oral fungi colonization did not show any significant decrease in renal transplant patients (RTPs) using miswak than in those not using miswak. This may be explained by the fact that all patients were receiving immunosuppressants and were medically stable. Probably, immunosuppressive drugs increase the propensity to oral fungal colonization (Al-Mohaya et al., 2002). However, prevalence for oral candidiasis was significantly lower in RTPs using miswak (Al-Mohaya et al., 2002).

This antimycotic effect is probably due to one or more of the miswak contents which include chlorine, trimethylamine, and alkaloids, resin, sodium chloride, potassium chloride, salvadoure, sulphur compounds, and oleic and linoleic acids (Al-Bagieh et al., 1994; Al-Mohaya et al., 2002; Al-Bagieh and Almas, 1997; Almas, 2002; Almas et al., 1997; Almas and Al-Lafi, 1995; Darout et al., 2000). These results indicate that extracts of miswak may contain compounds with therapeutic potential against different Candida strains, and hence, their possible use as therapeutic agents (Noumi et al., 2010).
Effect on human monocytes

In addition to the antimicrobial activity, miswak has protective effect on the human leukocytes. Its extract shows protective results on human monocytes from leukotoxin-induced cell lysis. Pre-incubation of monocytes with miswak extract inhibits leukotoxin-induced cell lysis when monocytes were exposed to leukotoxin (10 ng/ml) and even when the extract was removed before addition of toxin. This testifies a defensive effect on the target cell rather than an effect on the bacterial toxin. The leukotoxic activity of A. actinomycetemcomitans can significantly be abolished in the sample with miswak extract (Al-Otaibi et al., 2004). Further studies need to be done that which component of miswak is actually responsible for acting against leukotoxin.

Effect on Salivary pH

Studies have reported the preventive effect of miswak on dental caries and periodontal disease is due to its antibacterial effects, inhibitory action on dental plaque formation and mechanical toothbrushing (Almas et al., 2005). In addition to this, miswak extract has a neutralizing effect by raising the plaque pH after a previous sucrose exposure, which plays a synergistic effect in caries prevention. Miswak extract also stimulates parotid saliva flow rate significantly after the rinse. It may be due to the strong taste of the miswak extract and salivary stimulating oils for e.g. eugenol, thymol etc. However, rinse with freshly prepared aqueous miswak extract immediately raises the plaque pH and becomes most prominent after 30 minutes and persists up to 1 hour, proposes a mechanism other than increased salivary flow alone. This elevation in plaque pH may also be attributed due to an antibacterial effect. More research work is required to confirm these results and to explore and identify the underlying mechanisms of action, which take account for the confounding effects of mastication and normal salivary buffer capacity by using control group chewing placebo material e.g. paraffin tablet (Sofrata et al., 2007).

Antiplatelet aggregation effect

Miswak extract contains four benzylamides, namely- N,N-Bis(phenethyl)-2(S)-hydroxy-butanediamide, including N-benzyl-2-phenylacetamide, N-Benzylbenzamide and Benzyl urea. N-benzyl-2-phenylacetamide reveals a significant inhibitory effect on platelet aggregation induced by thrombin and collagen. Although the exact mechanism is not known, it is possibly attributed to suppression of cyclooxygenase (COX) or thromboxane synthetase (TXS) (Kuo et al., 1995). This suppression of COX and TXS by N-benzyl-2-phenylacetamide may possibly contribute to the anti-inflammatory action and provide foundation for its conventional use (Gleitz et al., 1997; Khalil, 2006).

Effect on cell proliferation and wound healing

Miswak increases cell proliferation and shows gum stimulating effects (Monks et al., 1990). The effect of miswak aqueous extracts was assessed on cell proliferation of the Balb/ C 3T3 mouse fibroblasts using MTT (3-2,5-diphenyltetrazolium bromide) assay. Highly significant increase in cell proliferation was observed at all the concentrations of miswak extracts (Darmani et al., 2006). Sanogo et al. observed that the decoction of Salvadora persica possessed significant protective action against ethanol and stress-induced ulcers (Sanogo et al., 1999). Conversely, another study indicated that S. persica mouthwash (as low as 0.1%) showed a significantly high cytotoxic effect on wound healing cells e.g. macrophage, epithelial, fibroblast and osteoblast cells. It is observed that alkaloids and flavonoids are responsible for this cytotoxic activity. It was suggested that S. persica mouthwash should be avoided in patients having fresh oral wounds (Rajabalian et al., 2009). Vitamin C (one of the component of miswak) along with other components may participate in wound healing properties and other tissue response.

Analgesic activity, sedative and anticonvulsant

Alali recommended that the extract can be used effectively as a natural tool for teeth cleaning and as a natural analgesic for the disturbing toothache (Alali and Al-Lafi, 2003). Eugenol might be responsible for its analgesic effect. According to Monforte, et al., oral administration of lyophilized decoction of miswak desensitises the rats to external stimuli. Whereas, its oral and systemic administration, provides protection against pentylenetetrazol PTZ-induced convulsion, by increasing the latency period, dropping the number of animals that exhibited convulsions, declining the duration of convulsions and by reducing mortality rate. In addition, miswak (decocted and chloroform extract) administration also potentiates the pentobarbital-induced hypnotic effects in rats, showing dose independent decrease of the induction time and an extension of the sleeping time (Monforte et al., 2002). The exact mechanism is not known but it is hypothesized that miswak compounds like lignan glycosides, organic sulphur compounds and related urea alkaloids might be suggestive of these neurological effects (Kamel et al., 1992; Monforte et al., 2002; Ray et al., 1975).

Effect on fertility

Another study investigated the toxic effects miswak extract on the reproductive system of the mouse. It caused a significant increase in uterine weights and a decrease in the relative ovarian and body weights. Darmani et al. hypothesized that reduction in ovarian weight might be due to interference with estrogen secretion as a result of a disturbance of the reproductive endocrine functions. It may also lead to enhanced secretion of progesterone, which is needed for endometrial alteration at the time of implantation and necessary for successful pregnancy. Furthermore, the relative weights of the testes and preputial glands were significantly increased and the seminal vesicles were significantly decreased in test males. This study also demonstrated that the number of pregnancies in females impregnated by test males was...
significantly declined than those impregnated by the control males. The alteration in the weight of accessory gland might suggest a change in the pattern of testosterone secretion. These results indicate that miswak effects adversely on male and female reproductive system and fertility (Darmani et al., 2003). These results indicate that more studies are required to better understand the mechanism and components of miswak responsible for fertility effect.

DISCUSSION

Miswak shows various beneficial biological effects like antibacterial, antifungal, antiplatelet aggregation, anticonvulsant effects, but the level of these effects varies in terms of the potency. Different levels of these beneficial effects attribute to difference in the types, methods, and concentrations of the Miswak extract. Additionally, it also depends on the absence or presence and the amounts of the active components in the miswak extract. The different methods of preparation may result in activation or deactivation or loss of some active components, with a marked enhancement or reduction of beneficial effect of that particular compound. Similarly, different concentration of the extract may increase or decrease the actual level of the active component and eventually influence the potency of the effect. Standard methods of extraction and concentrations should be used in future research to evaluate beneficial effects of the miswak components. Some studies have suggested volatile oils as active components, which requires selecting appropriate method of extraction to get all active miswak extract including the volatile oils. Future studies can be carried out to test the biological activity of miswak oil by using steam distillation as a possible method of choice to obtain essential volatile oil from the roots, stems, and leaves of *S. Persica* (Sofrata et al., 2008).

Another important aspect to improve the research on miswak is to identify specific active components of the extract for specific biological activity e.g. antibacterial, antifungal, antiplatelet activity, wound healing etc. For instance, the anticonvulsant and sedative activity of miswak has been demonstrated, but further studies need to be conducted about the compounds responsible for this and to know their neuropharmacological activity. Studying the properties of different miswak active compounds and their reactions to surrounding environments is also very important to choose the best available method for testing their oral health benefit activity. (Monforte et al., 2002; Sofrata et al., 2008)

However, very little information is available about the possible adverse effects on various body systems of its chronic use. Different compounds release during chewing and brushing process of miswak. These components can enter into the body while swallowing the saliva or into the circulatory system through inflammatory lesions in the gums. Using miswak for a long time could lead to increased levels of these components in the body. Darmani *et al.* conducted a study to find the adverse effects of miswak extract on reproductive system by administering (800 mg/kg) it intra-gastrically in rats (Darmani et al., 2003). It is almost impossible to reach such a high dose in stomach while brushing with miswak. Further studies need to be done to know how many times should miswak be used for brushing to reach its toxicity level that would affect different body systems in humans. Well-standardised studies are needed in the future to strengthen the evidence for miswak use.

CONCLUSION

*S. Persica* is a very useful and common natural source of cleaning oral cavity in the prevention of tooth decay and periodontal diseases. Besides this, it also shows various other biological effects. Despite this fact, not much work is done on this plant and not intensively studied. Further standardised studies need to be done to know its therapeutic potential and the mechanism and compounds involved.

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Biological effects of miswak


