Effect of aqueous extract of miswak on the in vitro growth of *Candida albicans*

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Abstract

Chewing sticks (miswak) which are the roots of *Salvadora persica* plant have been used for centuries as oral hygiene tools in many parts of the world particularly in Saudi Arabia. Many studies have demonstrated the antiplaque, antperiopathic, anticaries and antibacterial effect of these sticks. This study was designed to investigate the anticytic effect, if any, of the aqueous extract of the plant roots. Various concentrations of aqueous extract of miswak prepared with Sabouraud medium were inoculated with *Candida albicans* (oral isolate). These were incubated at 37°C and the turbidity was determined by OD at 600 nm wavelength measured at specific intervals over a period of 48 h. Data show that the extract at a concentration of 15% and above, has a fungistatic effect for up to 48 h. This anticytic effect was probably due to one or more of the root contents which included chlorine, trimethylamine, an alkaloid resin, and sulphur compounds.

Introduction

The relative accessibility and the popularity of chewing sticks in the Middle East and Africa as an oral hygiene tool make it a very cost-effective agent for plaque control in such communities (Elvin-Lewis 1979, 1980; Enwonwu and Amyanwu, 1985; Eid et al., 1990). Many studies have demonstrated the antibacterial, anticaries, antperiopathic and antifungal properties of aqueous extracts of various African chewing sticks (Wolinsky and Sore, 1983; Gazi et al., 1987; Rotimi and Mosadomi, 1988; Salako, 1990).

*Candida albicans* is responsible for severe chronic stomatitis seen in some denture wearers and its attachment to the denture base has been the subject of many studies (Budtz-Jorgensen, 1974; Theilade and Budtz-Jorgensen, 1988). Miswak chewing sticks have been used for oral hygiene in many North African and Middle East countries for a long time (Khoory, 1983). Investigations have shown that miswak possesses potent antiplaque and antibacterial properties (Elvin-Lewis, 1980; Gazi et al., 1987; Eid et al., 1990). However, a review of the literature has shown that no previous study has been done on its possible antifungal properties. The present investigation attempts to evaluate the anticytic properties, if any, of the miswak chewing stick.
Materials and methods

Preparation of miswak chewing stick

About 100 g of miswak chewing sticks were shaved with a sharp knife and powdered with a commercially available food blender. The powder was extracted with 50 ml of sterile distilled water and left at room temperature for 48 h. The supernatant was carefully decanted and centrifuged at 10,000 rpm for 15 min. The resultant supernatant was decanted and stored at 4°C until use (Salako 1989). The maximum storage period before use did not exceed 1 week (Rotimi et al., 1987) after which fresh extracts were made. Before use the required quantity of the extracts was filter-sterilized with a 0.22 μm FSFT membrane filter system from the Millipore Corporation, Bedford, Massachusetts, U.S.A. (Salako, 1989).

Preparation of C. albicans

Stock oral isolates of Candida albicans were used in this study. For the experimental work, primary cultures were grown on Sabouraud dextrose broth (SAB) at 37°C in an aerobic incubator for 24 h. The culture was diluted with sterile Sabouraud broth to achieve an optical density of 0.2 at 560 nm wavelength with a spectrophotometer. This was used as the standard inoculum throughout the study.

Microbiological assays

Various concentrations of the filter-sterilized miswak extracts ranging from 2.5—20% (v/v) in duplicates were prepared with sterile SAB. The standard inoculum (100 μl) was added to each of the tubes and the OD reading at 560 nm wavelength was immediately taken. Controls were treated similarly but without the extract. The OD readings were subsequently recorded at 3, 6, 9, 12 and 36 h, respectively. At 24 and 36 h, 100 μl aliquot (in duplicates) of each concentration and control sample were plated on Sabouraud glucose agar and incubated at 37°C for 24 h to correlate turbidity with the growth rate of the organism.

Results

Antifungal properties, which were concentration dependent, were evident when an aqueous extract of miswak was analysed. At 12 h after incubation, growth inhibition was observed in tubes containing extracts with concentrations of 5% and above, and at 24 h post-incubation inhibition was revealed in extracts of 7.5% and above. However, by 36 h only concentration levels of 15% and 20% produced significant inhibition of growth (Table 1). The results of the cultures from various tubes on Sabouraud glucose agar are shown in Figures 1a to 1d. These visual assessments confirmed the OD readings which showed that the greater the concentration of the extract the greater was the growth inhibition and the longer the period of such inhibition.
Table 1 Optical density readings obtained during growth of *C. albicans* in the presence of various concentrations of aqueous extract of miswak

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<th>Concen (%, v/v)</th>
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*Each concentration of the extract was tested using duplicate tubes (i.e. tube 1 and tube 2).*
Figure 1a  Effect of 5% miswak extract on colony forming units (CFU) of *C. albicans* after 24 h of incubation.

Figure 1b  Effect of 10% miswak extract on CFU of *C. albicans* after 24 h of incubation.
Figure 1c  Effect of 20% miswak extract on CFU of *C. albicans* after 24 h of incubation.

Figure 1d  Control plate. CFU of *C. albicans* in the absence of miswak extract after 24 h of incubation.

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Discussion

*C. albicans* is the most common oral fungus and it is estimated that up to 50% of normal healthy adults carry this yeast as a component of the normal oral flora (Arendorf and Walker, 1980). In most individuals, a competent immune system and the presence of a competing bacterial flora in the mouth, usually keeps this fungus under control (Allen, 1992). However, the organism causes a opportunistic infection when the host immune system is impaired either due to debilitating diseases such as leukaemia, diabetes and AIDS or prolonged use of certain drugs such as corticosteroids, antibiotics and some tranquilizers.

The clinical conditions of such infections range from pseudomembranous candidiasis (thrush) to a chronic atrophic variety usually seen in denture wearers (Palmquist *et al.*, 1984; Budtz-Jorgensen *et al.*, 1975). Furthermore, *C. albicans* has been associated with caries activity and its presence in large numbers may indicate the development of an acidic environment in the oral cavity (Krasse, 1954; Larmas, 1985).

Treatment of *C. albicans* infection is usually biomodal (Jacopino, 1992). The first stage is to remove the source and the next stage is to eliminate the infection from the tissues. One method of eliminating the source of infection is the use of denture cleansers. Such agents include alkaline peroxides, hypochlorites, acids, disinfectants and enzymes (Nakamoto *et al.*, 1991).

Results of the present investigation suggest that aqueous extracts of miswak could be used to reduce growth of *C. albicans*. Such inhibition lasted for up to 36 h at concentrations of 15% and above. The mechanism by which this extract produced such inhibition was not investigated. However, previous work on many chewing sticks used in different parts of the world have shown that they contained alkaloids, and tannin-like substances, which are known to have antibacterial properties (Khoory, 1983; Wolinsky and Sote, 1984). This was further corroborated by Kubota *et al.* (1988) who found a reduced *C. albicans* attachment to denture bases treated with tannic acid.

Chemical analysis of miswak extracts showed that it contained chlorine, trimethylamine, an alkaloid resin, silica, vitamin C, sulphur and tannins (Elvin-Lewis, 1979). These may be responsible for the antibacterial and the antifungal activities of the chewing stick. However, the clinical efficacy of the extracts deserves further study because many of the antimicrobial substances and chemical denture cleansers currently available in the management of *C. albicans* carry with them some degrees of side effects and other disadvantages (Nakamoto *et al.*, 1991). Furthermore, in countries where chewing sticks are still used for oral hygiene, extracts from them would be acceptable culturally for oral rinsing purposes and as denture cleansers.

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