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A Comparative study of *invitro* antibacterial activity of neem and miswak extracts against isolated cariogens from dental caries patients

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ABSTRACT

The chewing sticks have been in tradition for several decades without knowing the scientific value of it. The aim of the present study was to make a comparative study about the antibacterial activity between neem and miswak in dental caries patients with diabetes. About fifteen diabetic patients with chronic dental caries were selected on the basis of information through a questionnaire form. The saliva and the plaque samples were collected from them. The effectivity of caries was determined by the caries susceptibility test. Streptococcus sp. and Lactobacillus sp., the predominant cariogens were isolated from them and the antibacterial activity of aqueous extract of neem and miswak at 50% concentration proved to exhibit maximum antibacterial activity.

Key words: Cariogens, chewing sticks, miswak, gingivitis.

INTRODUCTION

People with diabetes have an increased risk of caries, a demineralization of the hard tooth structure (enamel, dentin) due to a bacterial infection. This could be a direct consequence of hyperglycemia [9]. The oral health of diabetic patients has been the subject of many studies in recent years. While these patients are acknowledged to have an increased susceptibility to periodontal disease, the probability of detecting an increased prevalence of dental caries in patients with type 1 diabetes is more controversial [13,21 23]. Dental caries is one of the most

important problems in public health because of its ubiquitousness in civilized populations. The prevalence of dental caries in industrialized countries like India is on the rise [18]. Mutans Streptococci is the most cariogenic pathogen as it is highly acidogenic, producing short chain carboxylic acids which dissolve hard tissues such as enamel and dentin [3, 25]. Dental caries is a multifactorial infectious disease, related to biofilm accumulation on dental surface and frequent consumption of fermentable carbohydrates [6]. The microbial flora of the mouth is highly complex, containing a wide variety of bacterial species. The most common types of oral diseases, such as dental caries and periodontal diseases are both related to dental plaque and seem to occur when the normal balance between the microorganisms and the host is disturbed in some way [4]. Traditional systems rely heavily on herbal materials compared to modern system of medicine [24]. Many plants were reported to inhibit the growth of many oral bacteria particularly, *Streptococcus mutans* [10]. Many side effects have been reported in chemical mouth rinses. The incidence of side effects such as undesirable tooth discoloration, unpleasant taste, dryness and burning sensation in the mouth discourage patients to use this mouthwash. Recently, the use of herbal mouth washes is also increasing [20]. Chewing sticks were used by the Babylonians some 7,000 years ago. They were later used by the Greek and Roman Empires and had been used by Jews, Egyptians. Today, they are used in Africa, Asia, the Eastern Mediterranean region and South America [13]. Herbal medicine has such an extraordinary influence that numerous alternative medicine therapies treat their patients with Herbal remedies, Unani and Ayurveda [19]. The World health organization (WHO) [11] has recommended and encouraged the use of chewing sticks. Chewing sticks have been shown to have a therapeutic effect on the gingiva and surrounding structures in addition to their mechanical effect [16]. There are various plants which are used as chewing sticks the lime tree (*Citrus aurantifolia*) and the orange tree (*Citrus sinensis*) are sometimes used as chewing sticks. The roots of Senna (*Cassia vinnea*) were used by American Negroes and those of African laburnum (*Cassia sieberiana*) were used in Sierra Leone. Neem (*Azadirachta indica*) widely used to provide chewing sticks in Indian subcontinent [1, 7]. The neem is of particular interest to the field of dentistry for it has a long history treating teeth and gum problems. In rural areas of India, the twigs are used as toothbrush to prevent gingivitis [19]. The neem tree (*Azadirachta indica*) has the widest spectrum of use of all natural products. The first known use of neem by the Harappa culture in ancient India dates back 4500 years [14]. Similarly another tooth stick name miswak is also widely used as a chewing stick in many countries. The toothbrush tree, *Salvadora persica*, L., locally called miswak, is a member of the Salvadoraceae family has been used by many Islamic communities as toothbrushes and has been scientifically proven to be very useful in the prevention of tooth decay, even when used without any other tooth cleaning methods [8].

EXPERIMENTAL SECTION

Isolation and identification of cariogens from dental caries patients:-

The saliva and the swab from plaque lesions were collected from the diabetic patients with dental caries and transported to the laboratory. The saliva was plated on the plate count agar for the enumeration of total count of bacteria. The swabs collected from the patients were plated on brain heart infusion agar maintained in anaerobic jar for the anaerobic organisms growth for 2-3 days. The colonies from the BHIA were examined and plated on special media like mitis-salivarius agar and Rogosa agar for detecting the most cariogenic family members like *Streptococcus sp.* and *Lactobacillus sp.* and then other confirmatory biochemical test were

performed. The identification procedure was done with the proceedings of Cowans and Steel method.

Preparation of aqueous extract of chewing sticks:-

About 100gm of each chewing sticks (*Azadirachta indica* and *Salvadora persica*) were used in the experiment. The chewing sticks were kept sun dried for two weeks at before extract preparation. The characteristic feature of neem and the miswak parts were given in table I. The sticks were cut into small pieces and ground to powder in a mixer, the powder was made juice by adding sterile double distilled water in the ratio of 1:2. Then it was squeezed in sterile gauze and the extract was collected by hand pressure. Then this supernatant was filtered through membrane filter, having a pore size of 0.45micrometer. The filter apparatus was sterilized before filtration by autoclaving. A sterile nutrient agar slant was inoculated with the filtered aqueous for checking the sterility of extract. The extract was distributed in screw capped bottles and stored in deep freezer at -20°C until used.

Determining the antibiogram activity of Miswak and Neem:-

The ditch plate method was used to test the antibacterial activity. Sterile Muller-Hinton agar was prepared and poured into petridishes. After solidification 6mm ditches were made. Into the ditches made at the centre of petridishes, 0.1ml aliquots of the miswak and neem extract were pipette out at various concentrations like 10%, 20%, 30%, 40% and 50%. The plates were left for 1 hour at room temperature and then incubated anaerobically at 37°C for 48 h and examined for inhibition [1]. Standard 6-mm discs containing streptomycin (25 µg/disc) was used as a control.

Caries susceptibility test:-

A significant correlation was noted between the amounts of *Lactobacillus sp.* in saliva and individual susceptibility to dental caries. *Lactobacilli sp.* population may be obtained by incubating saliva samples in a selected medium and noting the rate of acid production as it relates to incubation time, this procedure was known as Snyder's test after its developer, Marshall L. Snyder. Caries susceptibility test is based on growth of *Lactobacilli sp.* in Snyder's test agar, a medium that contains glucose and an indicator bromocresol green. The indicator appears as green in pH of 4.8 and yellow at pH 4.4 and below. As *Lactobacillus sp.* grows and multiplies, the acid they produce causes pH to drop and color to change [2].

Preparation of Snyder test medium:-

The tubes of melted Rogosa SL agar were prepared. Aseptically a loopful of saliva was inoculated into a deep tube of melted Rogosa SL agar. The bottom of the tube was tapped to thoroughly mix the contents and agar was poured into the Petri dishes. The agar was allowed to harden thoroughly; the plates were inverted and incubated at 37°C for 24-48 hours at anaerobic condition. Plates were observed for of well isolated colonies of *Lactobacillus*. By referring to the standard table II, an estimate of *Lactobacillus sp.* population was made and caries susceptibility test was determined.

RESULTS

Total enumeration count of bacteria

The total counts of bacteria were enumerated from the fifteen dental caries person in this study. The total count of bacteria ranges from 62.9×10^6 to 101×10^6

Isolation and identification of dental caries causing bacteria

Lactobacilli sp. and alpha hemolytic *Streptococci sp.* were isolated the dental caries persons. Among this *Streptococcus sp.* were reported from twelve persons among fifteen investigated persons and the *Lactobacilli sp.* were isolated about from nine patients. The characteristic identification of *Streptococcus sp.* was shown in table.III.

Invitro antibacterial activity of extracts of neem and miswak

A considerable antibacterial activity of the aqueous extract on *Streptococcus sp.* and *Lactobacillus sp.* was noticed at higher concentration of both *Azadirachta indica* and *Salvadora persica*. The maximum inhibitory antibacterial activity of the aqueous concentrations of *Azadirachta indica* and *Salvadora persica* were noted at about 50% concentration as 16mm and 15mm for *Streptococcus sp.* were as for *Lactobacillus sp.* its of 14 mm and 16 mm respectively. The antibiotic disc streptomycin shows inhibition of about 17mm to *Streptococcus sp.* where as for 19mm for *Lactobacillus sp.* as given in table IV.

Caries susceptibility test

Among the fifteen patients in this study, about twelve patients showed slight activity where as only about three patients showed moderate activity as shown in table V.

DISCUSSION

The microorganisms associated with dental caries were studied in diabetic patients .It reported that a high proportion of *Streptococcus mutans* in the oral cavity [20]. Diabetic patients are known to tend to develop caries in concrete locations. In this sense, saliva has been identified as a factor related to the appearance of dental neck caries [9]. In this study also, saliva and caries lesions sample were collected from the diabetes patients who were identified with dental caries. *Streptococci* species were isolated, identified and counted in 262 saliva samples collected from 131 children and adolescents from a public school nursery at the city of Jaboticabal [17, 25]. In the present study among the fifteen patients *Streptococcus sp.* was isolated from twelve patients but *Lactobacillus sp.* was isolated from only nine patients. For the cultivation of lactobacilli, the dilutions were spotted on the dried surface of a Rogosa SL (Difco B480) agar plate [26]. According to WHO nearly 80 % of the global population still relies upon the herbal drugs for their primary health care [27, 13]. So in this study too, two herbal plants were taken to evaluate their antimicrobial activity against cariogens. The neem leaves extract has an antibacterial activity towards *Streptococcus mutans* [18].The aqueous extract of neem was reported to contain several active substances and is known to posses anti-inflammatory and immunomodulatory activites. [25, 26]. Similarly, in this study the stem extracts of neem showed antibacterial activity against the isolated cariogens.

The antibacterial activities of miswak in the present study were similar to the results of [1, 21] of which the extracts have a good inhibitory effect against the *S.mutans*, the present also denoted a satisfactory inhibitory result of miswak against *Streptococcus sp.*

The antibacterial activity of aqueous extract of miswak may be attributed to the presences of various chemicals in its extracts such as sodium chloride, potassium chloride as well as salvadourea, salvadorine, saponins, tannins, vitamin C, silica and resin [12].The maximum

inhibitory antibacterial activity of the aqueous concentrations of *Azadirachta indica* and *Salvadora persica* were noted at about 50% concentration as 16mm and 15mm for *Streptococcus sp.* were as for *Lactobacillus sp.* its of 14 mm and 16 mm respectively. The antibiotic disc streptomycin shows inhibition of about 17mm to *Streptococcus sp.* where as for 19mm for *Lactobacillus sp.* In the effects of *Salvadora persica* and Padina essential Oil solution in comparison to chlorhexidine in human gingival disease have benefits [16].

Table I: Characteristics of *Azadirachta indica* and *Salvadora persica* extracts

S.no.	Plants	Plant part used	pH of extract	Colour
1	Neem (<i>Azadirachta indica</i>)	Stem	6.8	Brown
2.	Miswak (<i>Salvadora persica</i>)	Stem	6.7	Straw colored

Table II: Standard table for Caries susceptibility test

Caries susceptibility	Time for medium to turn yellow		
	24 hours	48 hours	72 hours
Marked	+	-	-
Moderate	-	+	-
Slight	-	-	+
Not susceptibility	-	-	-

Table III: Isolation and identification of Cariogens

Characteristics	<i>Streptococcus sp.</i>	<i>Lactobacillus sp.</i>
Growth anaerobically	Positive	Positive
Growth on blood agar	Alpha haemolysis	-
Growth on special media: On Mitis-salivarius agar	Small blue colour colonies	-
On Rogosa agar	-	Small white colour Colonies seen
Gram's staining	Gram positive cocci in pairs or chain	Gram positive rods
Catalase	Negative	Negative
Oxidase	Negative	Negative
Starch hydrolysis	Negative	Negative
Esculin hydrolysis	Positive	Positive
Hippurate hydrolysis	Negative	Negative
Arginine hydrolysis	Negative	Negative
Bacitracin sensitivity test(0.1 unit)	Negative	Negative
Optochin sensitivity test	Negative	Negative
Fermentation test:		
Inulin	Positive	Negative
Lactose	Positive	Positive
Arabinose	Negative	Negative
Raffinose	Positive	d
Trehalose	Positive	d
Sorbitol	Positive	Negative
Ribose	Negative	Negative

Table IV: Antibacterial activity of chewing sticks

S. no	Aqueous extract of the stem	Isolated organisms	Solvent extracts used	Maximum zone in diameter (mm)
1	<i>Azadirachta indica</i>	<i>Streptococcus sp</i>	10%	-
			20%	-
			30%	10
			40%	12
			50%	16
		<i>Lactobacillus sp</i>	10%	-
			20%	10
			30%	11
			40%	12
			50%	15
2	<i>Salvadora persica</i>	<i>Streptococcus sp</i>	10%	-
			20%	-
			30%	10
			40%	12
			50%	14
		<i>Lactobacillus sp</i>	10%	-
			20%	-
			30%	11
			40%	12
			50%	16

Table V: Caries susceptibility test

S.no	Study group persons	Colour of Snyder test			Caries susceptibility
		24 hours	48 hours	72 hours	
1	C1	Green	Green	Yellow	Slight
2	C2	Green	Green	Yellow	Moderate
3	C3	Green	Green	Yellow	Slight
4	C4	Green	Green	Yellow	Slight
5	C5	Green	Green	Yellow	Slight
6	C6	Green	Green	Yellow	Slight
7	C7	Green	Green	Yellow	Slight
8	C8	Green	Green	Yellow	Moderate
9	C9	Green	Green	Yellow	Slight
10	C10	Green	Green	Yellow	Slight
11	C11	Green	Green	Yellow	Slight
12	C12	Green	Green	Yellow	Slight
13	C13	Green	Green	Yellow	Slight
14	C14	Green	Green	Yellow	Slight
15	C15	Green	Green	Yellow	Moderate

Present data indicates that the antibacterial activity of aqueous extracts of neem and persica have a remarkable activity against the isolated cariogens. Along with normal oral hygienic practices like tooth brushing, cleansing it can also be recommend chewing the natural sticks like neem and persica to get better results in oral health especially in diabetic patients.

CONCLUSION

The prevalence of dental caries in diabetes mellitus patients in most of the industrialized and developing countries is on the rise. As the treatment is very costly for advanced dental problems, the prevention at the primary level is the solution of choice. Natural products have been used for thousands of years in folk medicine for several purposes. As most of the oral diseases were due to the bacterial infections, it has been well documented that medicinal plants confer considerable antibacterial activity against various microorganisms including bacteria responsible for dental caries. Based on our results, we conclude that the use of neem and persica as either as chewing sticks or else in other forms like mouth washes will be effective with less side effect especially in diabetes patients since they have many medications for their health concern.

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