Research Article

Available online through
www.ijrap.net

IJRAP
ISSN 2229-3566

QUALITATIVE EVALUATION OF SUBGINGIVAL MICROFLORA AFTER THE CHEWING OF BETEL LEAF

K Vani[1], Jose Maji*[2], Rao Srinivasa[3]

[1]Dept. Of Oral Pathology & Microbiology, Century International Institute of Dental Sciences & Research Centre, Poinachi, Thekkil, Kasaragod, Kerala, India

[2]Dept. Of Oral Pathology and Microbiology, Yenepoya Dental College, Yenepoya University, Mangalore, India

[3]Dept. of Oral Surgery, Century International Institute of Dental Sciences And Research Centre, Poinachi, Thekkil Kasaragod, Kerala, India

Received on: 19/06/2011 Revised on: 29/07/2011 Accepted on: 02/08/2011

ABSTRACT

Betel a branching vine, scientifically called as *Piper betel,* is used in a number of traditional remedies and known to have immune boosting as well as antibacterial properties. This study was conducted to assess the qualitative changes in the sub-gingival micro flora, after the chewing of betel leaves, in order to evaluate the antimicrobial effect of this medicinal plant on oral pathogens.

Forty volunteers were made to chew betel leaves daily for 5-10 minutes for a period of two weeks and the sub gingival plaque samples were analyzed to identify the change in type organisms if any.

We have identified a wide variation in the type of organisms in the sub gingival plaque samples after the study period. Many organisms had reduced while few increased and interestingly few organisms which were not observed in the plaque sample prior to the use of betel leaf have appeared after the use. Significant alteration observed was pertaining to *Streptococcus viridans* group of organisms which is the commonest oral pathogen.

The analysis showed chewing of betel leaf can reduce the pathogenic organisms in the sub gingival microflora.

KEY WORDS: Oral Microbes; Oral Habits; Betel Leaf Chewing; Anti microbial property; *S.Viridans*

***Author for Correspondence**

Dr. Maji Jose, Professor and Head, Dept of Oral Pathology & Microbiology, Yenepoya Dental College, Nithyananda Nagar, Derlakatte Mangalore-575018, Karnataka State, India Email – majiajoyin@yahoo.co.in

INTRODUCTION

Betel is a branching vine which is scientifically called as *Piper betel* and belongs to the family Piperaceae[1]. The custom of chewing 'betel' is an ancient one, extending back in time at least several centuries B.C. It is linked with a number of social traditions. Some evidence suggests that betel leaves have immune boosting properties as well as anti-cancer and antibacterial properties. Various constituents of betel appear to have antiseptic, bactericidal and antioxidant effects[1]. Though the custom of chewing 'betel' is an ancient one, the effect of the same on the oral flora is not freely available in literature and possible antimicrobial properties of this valuable plant on oral pathogens are yet to be explored in detail. The present work is conducted to study the effect of betel leaf chewing on sub gingival microflora.

MATERIAL AND METHODS

The present study was conducted in Yenepoya Medical and Dental College campus, Deralakatte, Mangalore. Study sample consisted of forty healthy adult volunteers, including males and females of an age range of 20-30 years.

Only healthy individuals without any adverse oral habits such as tobacco smoking or chewing were considered for the study. After thorough oral examination, gingival health status was assessed using the gingival index of Loe and Sillness. Subjects with gingival index score of 0.1 – 1 i.e. individuals with sound gingival status with no or mild gingival inflammation were finally selected to participate in the study. After obtaining the written consent, sub gingival plaque samples were collected from the gingival sulcus of mandibular molar teeth and subjected to microbial analysis, to determine the types of microorganisms.

At the commencement of the study, a thorough oral prophylaxis was done for every participant and oral hygiene instructions were given. Furthermore to ensure that all the participants follow the same oral hygiene procedure, they were instructed to use a standard tooth paste which was given to them. One week after the oral prophylaxis, the plaque sample was collected.

During the first phase of the study, before the participants started chewing betel leaves, they were asked to chew a pliable inert material (paraffin wax of standard size) every day for 5-10 minutes for a period of two weeks. This was done to evaluate the effect of mechanical cleansing on sub gingival flora. At the end of phase one, sub gingival plaque samples were collected from gingival sulcus of mandible molar teeth and subjected to microbial analysis.

During the second phase of the study, the same participants were asked to chew betel leaves every day for 5-10 minutes a period of next two weeks. Betel leaves were collected from local sellers and were provided to the participants. Each day one betel leaf of standard size was rolled and then chewed for five to ten minutes. At the end of two weeks, sub gingival plaque samples were collected from the same region and subjected to microbial analysis.

Thus the microbial qualitative analyses of sub gingival plaque were carried out three times during the study period and were named as first, second, and third microbial analyses. Microbial examination was carried out in the Department of Microbiology Yenepoya Medical College, Mangalore. The standard methods for isolation, biological characterization and identification of microorganisms followed for aerobic and anaerobic bacterial colonies were as per Cowen and Steel[2].

The results of the first, second and third microbial analysis were analyzed taking the percentage of volunteers showing the alteration of the organisms.

RESULTS

There was considerable difference in the composition of sub-gingival plaque after chewing of betel leaf for two weeks. The qualitative analysis of sub gingival plaque showed presence of *Streptococcus viridans* in the oral flora of majority of volunteers (97.5%) and a significant reduction(Table 1) from first to second to third microbial analysis, 97.5%, 93.5%, 9.7% volunteers respectively. Similarly Coagulase negative *Staphylococcus* was also found to be decreasing after the chewing of betel leaves although these were increased from first to second microbial analysis. Other organisms like *Candida albicans, Branhamella* species and *Micrococci* were also decreased in some percentage of volunteers.

The species like *Nocardia*, *Lactobacilli*, *Neisseria*, *Pseudomanas* and *Veillonella* were found to be increased in number after chewing betel leaf(Table 2).

At the same time, other species like *Enterobactor*, *Actinomyces*, *Camplytobactor*, *Prevotella* species, *Streptococcus fecalis* which were not present in first and second microbial analysis have showed a growth in third microbial analysis of 12.5%, 7.5% 10%, 7.5% of the volunteers respectively(Table 3).

DISCUSSION

Traditional or alternative oral hygiene measures are gaining importance among general public. Studies were conducted on many such measures to assess the antimicrobial efficacy with varied success. The substances used in these studies were Mango leaf, Tea tree oil, Neem leaf, Garlic[3, 4]. Similarly betel leaf is a branching vine, used widely in traditional medicine. Betel leaf is an important ingredient of betel quid, chewing of which is a common practice in the Indian subcontinent[1]. Betel leaves are used as a stimulant, antiseptic and breath-freshener in traditional medicine and are known to have immune boosting properties as well as antibacterial properties and anticancerous properties[1]. The present study was conducted to assess the qualitative changes in oral microflora after the use of betel leaf. It is chewed in the form of quid, the composition of which varies from place to place. Few studies are available on the effect of quid chewing on oral microflora.

The qualitative results of first microbial analysis of our study showed *Streptococccus viridans* in the sub gingival plaque sample of 97.5% of the volunteers. It is an established fact that *Streptococcus viridans* group form the predominant member of oral flora[5]. They have been isolated from all sites in the mouth and comprise a large proportion of the resident microflora. On the basis of biochemical reactions, Streptococcus *viridans* can be classified into four species: *Streptococcu salivarius, Streptococcus mutans, Streptococcus anginosus* and *Streptococcus mitis*[7]. These groups of organisms have been identified as a major group that causes dental caries. Frandes EVG, Pedrazzoli V, Kilian M [5], in their study on the ecology of *Streptococcus viridans* in the oral cavity and pharynx, confirmed and extended the earlier findings. They said that apart from mature supra gingival plaque, which contains a mixture of all orally encountered Streptococci, each site in the oral cavity shows a characteristic streptococcal flora.

Other species that were isolated in first microbial analysis in varying amounts were Coagulase negative *Staphylococcus*, *Nocardia* species. *Lactobacillus, Candida albicans, Neisseria* species, *Pseudomonas,*

Branhamella species, *Peptostreptococcus, Villanelle, Micrococci*, Anaerobic *Streptococci* and *Serratia*. Among these *Lactobacillus,* Coagulase negative *Staphylococci* and *Branhamella* species were observed in 30.0%, 17.5%and 25.0% of the volunteers respectively. Rest of the microorganisms was found only in less than 15% of the volunteers. Thus, similar to the previous reports we have also observed a wide variety of microorganisms at sub gingival site. The literature shows that gingival crevice is a distinct microbial habitat, influenced both by the anatomy of the site and flow and property of gingival crevicular fluid. This is reflected in the higher species diversity at this site [6, 7].

The second microbial analysis was done two week after chewing of paraffin wax. Paraffin wax is an inert material, chewing of which was advised before analyzing the effect of betel leaf on oral flora, in order to assess the effect of mechanical cleansing alone. After chewing of paraffin wax pellets the oral flora did not show a significant change from the first microbial analysis indicating that only mechanical cleansing property of a material, do not have much influence on sub gingival plaque microbial population. *Streptococcus viridans* were present in the majority of the volunteers (93.5%) even in the second microbial analysis. Some changes were observed between the first and second microbial analysis in organisms like *Candida albicans*, Coagulase negative *Staphylococcus* and *Branhamella* species which could be because of the constant state of fluctuation in the dynamic ecosystem of oral flora[7].

In the third microbial analysis i.e., the oral flora cultured after two weeks of chewing betel leaf showed a reduction of *Streptococcus viridans* in significant numbers of volunteers (9.7%), and also a reduction of Coagulase negative *Staphylococci, Candida albicans, Branhamella* species and *Micrococci* in some. Few microorganisms like *Enterobactor, Actinomyces, Camplytobactor, Prevotella* species and *Streptococcus fecalis* which were absent in first microbial analysis have shown growth in third microbial analysis in 12.5%, 7.5% 10%, 7.5% of the volunteers respectively.

Since we have ruled out the role of mechanical cleansing effect on sub gingival plaque organisms the significant reduction in *Streptococcus viridans* and also other organisms observed in our study could be because of the inhibitory effect of chemical constituents of betel leaf on these organisms. Since the oral flora is a dynamic ecosystem, the decrease in some bacteria would have lead to the increase in the growth of some new species which were not observed in the first two analyses.

To compare our observations, only few studies were available in the literature on the influence of betel quid chewing on oral microflora. One of the study conducted by Ling et al[8] reported that betel quid chewing increases the likelihood of sub gingival infection with *A. actinomycetumcomitans* and *P.gingivalis*. The study was conducted in Taiwan people where they used betel quid containing two halves of fresh areca nut sandwiched with a piece of inflorescence and with or without betel leaf. *Piper betel* inflorescence, which is used in some areas in the quid, contains 15.4 mg of safrole. An *in vitro* study was conducted on the effect of safrole on the defensive functions of human neutrophils and concluded that inhibition of defensive functions of neutrophils may be one possible mechanism by which safrole compromises the oral health[9].

This work is not an attempt to give a good image to betel quid chewing habit which is with tobacco. While accepting the potential adverse effects of betel quid chewing, we should objectively look at the beneficial aspects of betel leaf which has long been hosted by Indian traditional medicine. Of particular importance in this regards would be the possibility of isolating biologically active components that can be used therapeutically.

Having known the beneficial effect of these leaves, it will be relevant to conduct a further systematic study to discover the valuable chemical constituents that can be incorporated in to modern oral health care system

CONCLUSION

The study has confirmed the effectiveness of the betel leaves in reducing the pathogenic microbial organisms in the sub gingival flora. This could be due to the chemical constituents present in the betel leaf. The beneficial components need to be identified so that it can be used therapeutically.

ACKNOWLEDGEMENT

- Dr. Vidhya. M, Additional Professor, Dept. Of Oral Pathology And Microbiology, A.B. Shetty Memorial Institute Of Dental Sciences, Mangalore
- Mr. Mohan Kotian, Dept Of Microbiology, Yenepoya Medical College, Mangalore

REFERENCES

1. Guha P. Betel leaf: The neglected Green Gold of India. J Hum.Ecol 2006; 19:2: 87-93.
2. Barrow GI and Feltham R.K.A. Cowen and steel's Manual for identification of medical bacteria 3rd edition Cambridge: Cambridge university press 1993,171-174.
3. Hammer KA, Dry L, Johnson M, Michalak EM, Carson CF, Rilety TV. Susceptibility of oral bacteria to Melaleuca Alternifolia (tea tree) oil in vitro. Oral Microbiol Immunol 2003; 18: 389-392.
4. Nair R, Kalariya T, Chanda S. Antibacterial activity of some selected Indian medicinal flora. Turk J Biol 2005; 29: 41–47.
5. Frandes EVG, Pedrazzoli V, Kilian M. Ecology of viridans streptococci in the oral cavity and pharynx. Oral microbial Immunol; 6: 129-133.

6. Marsh PD. Are dental diseases examples of ecological catastrophes? Microbiology 2003; 148: 279-294.
7. Marsh PD, Martin MV. Oral Micribiology, 4th edition. Oxford: Wright 1999; 86-98
8. Ling LJ, Hung SL, Tseng Sc, Chen Ly, Wu KM, Lai YL. Association between betel quid chewing, periodontal status and periodontal pathogens. Oral Microbiol Immunol 2001; 16: 364-369.
9. Hung SL, Chen YL, Chen YT. Effect of safrole on the defensive functions of human neutrophils. J periodont Res 2003; 38: 130-134.

Table 1 The organisms which reduced after chewing betel leaves

Microorganisms	Percentage of volunteers showing the growth		
	First microbial analysis	Second microbial analysis	Third microbial analysis
Streptococcus viridans	97.5%	93.5%	9.7%
Coagulase negative *staphylococcus*	17.5%	30.0%	22.6%
Branhamella spp.	25.0%	32.5%	30.0%
Micrococci	5.0%	12.9%	9.7%
Candida albicans	7.5%	0.0%	5.0%

Table 2. The organisms which increased after chewing betel leaves

	Percentage of volunteers showing the growth		
Microorganisms	First microbial analysis	Second microbial analysis	Third microbial analysis
Nocardia spp.	7.5%	9.7%	12.9%
Lacto bacillus	30.0%	32.2%	45.2%
Neisseria spp.	12.5%	12.5%	25.0%
Pseudomonas	7.5%	7.5%	9.7%
Veillionella	5.0%	5.0%	7.5%

Table 3 -The organisms which appeared after chewing betel leaves

	Percentage of volunteers showing the growth		
Microorganisms	First microbial analysis	Second microbial analysis	Third microbial analysis
Enterobacter	0.0%	0.0%	12.5%
Actinomyces	0.0%	0.0%	7.5%
Camphylobacter	0.0%	0.0%	10.0%
Prevotella spp	0.0%	0.0%	10.0%
Streptococcus fecalis	0.0%	0.0%	7.5%

Source of support: Nil, Conflict of interest: None Declared