*British Microbiology Research Journal*
*9(5): 1-5, 2015, Article no.BMRJ.18568*
*ISSN: 2231-0886*

**SCIENCEDOMAIN** *international*
www.sciencedomain.org

# Smokeless Tobacco – Ammunition against Dental Caries? A Microbial Study

**Priya S. Joshi[1*], Madhuri Chougule[1], Shirish G. Joshi Reader[2], Bhagyalaxmi P. Hongal[1] and Rucha S. Kore[1]**

*[1]Vasantdada Patil Dental College & Hospital, Sangli, Maharashtra, India.*
*[2]Department of Microbiology, Vasantdada Patil Dental College & Hospital, Sangli, Maharashtra, India.*

***Authors' contributions***

*This work was carried out in collaboration between all authors. Author PSJ designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors MC, SGJR, BPH and RSK managed the analyses of the study and literature searches. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/BMRJ/2015/18568
Editor(s):
(1) Alok K Upadhyay, Fox Chase Cancer Center, Philadelphia, Pennsylvania, USA.
Reviewers:
(1) Anonymous, University of Maringá, Brazil.
(2) Anonymous, Universidade Estadual de Londrina, Brazil.
(3) Joshua Mbanga, Department of Applied Biology and Biochemistry, National University of Science and Technology, Zimbabwe.
Complete Peer review History: http://sciencedomain.org/review-history/10130

***Original Research Article***

***Received 29th April 2015***
***Accepted 23rd June 2015***
***Published 10th July 2015***

## ABSTRACT

**Aims:** The tobacco plant, *Nicotiana tabacum*, has been one of the major risk factors for oral cancer. However, the literature has endowed it as "holy herb" since the pre-Columbian era as being used for treating pain, poisonous bites, ulcers, nasal polyp. So we designed a study to elicit the anti-microbial property if any, in smokeless tobacco against *Streptococcus mutans* and also to study the relationship of growth inhibition efficacy of three forms of smokeless tobacco, namely, raw tobacco leaves, mishri and khaini.
**Study Design:** Cross-sectional, observational study.
**Place and Duration of Study:** Department of Oral Pathology and Microbiology, Vasantdada Patil Dental College and Hospital, Kavalapur, Sangli, Maharashtra, India, between December 2014 to February 2015.
**Methodology:** Twenty two ml of un-stimulated whole saliva was collected from each of five healthy subjects, with no habit of tobacco consumption in any form, zero DMFT index. The samples were

**Corresponding author: E-mail: sangeetakov@yahoo.co.in;*

collected in the morning, half an hour after tooth brushing and were stored at 4°C prior to processing. Extracts from three forms of smokeless tobacco namely raw tobacco leaves, mishri and khaini were evaluated. The tobacco extracts were prepared using saliva and ringer lactate which was previously sterilized. The antibacterial effect of tobacco extracts was evaluated by well-diffusion method using Brain Heart Infusion agar plates. The bacterial growth inhibition zones were measured after 24 hours incubation at 37°C. Ciprofloxacin was used as a positive control.

**Results:** All extracts exhibited an inhibitory effect on the growth of *S. mutans*, except mishri extract. The mean diameter of bacterial growth inhibition zones for khaini extracts against *S. mutans* was higher than those of raw tobacco leaf and mishri extracts. The differences in the inhibition zones were however not statistically significant (One way Anova, $p > 0.05$).

**Conclusion:** Amongst the three forms of tobacco extracts analyzed, khaini had the highest anti-microbial property against the growth of *S. mutans* as compared to raw tobacco leaves and mishri.

*Keywords: Dental caries; khaini; mishri; raw tobacco leaves; ringer lactate; saliva; Streptococcus mutans.*

## 1. INTRODUCTION

Dental caries is among the most important preventable infectious diseases worldwide. The association between dental caries and the oral microbiota is also well established [1].

*Streptococcus mutans* is closely associated with dental caries, mainly those involving smooth surfaces [2]. The primary habitats for *S. mutans* are mouth, pharynx and intestine. *S. mutans* possesses several inherent properties such as adherence to enamel surfaces, production of acidic metabolites, the capacity to build up glycogen reserves and the ability to synthesize extracellular polysaccharides. They have a central role in the etiology of dental caries because they can adhere to the enamel salivary pellicle and to other plaque bacteria. *S. mutans* are strong acid producers and hence cause an acidic environment creating the risk for cavities. Usually the appearance of *S. mutans* in the tooth cavities is followed by caries after 6-24 months [3].

Medicinal plants including tobacco have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world [1]. Smokeless tobacco is known to contain approximately 4,200 chemicals. Chemicals in smokeless tobacco include alkaloids such as nicotine, nor-nicotine, cotinine, anabasine, anatabine, aliphatic hydrocarbons, and hundreds of isoprenoids that produce typical aroma of tobacco leaves. A number of phytosterols such as cholesterol, campesterols and alcohols, phenolics, chlorogenic acid, rutin, carboxylic acids, turpenes, polyphenols, aromatic hydrocarbons, aldehydes, ketones, amines, nitrites, N- and O-heterocyclic hydrocarbons, pesticides, and alkali nitrates have also been detected. Toxic metals including mercury, lead, chromium, and other trace elements and several free amino acids are also present. Nicotine, the addictive substance exists in two forms- acid (bound) and base (free). Free or unionized nicotine is most rapidly and easily absorbed in the mouth. Slaked lime or other alkaline additives contribute to high pH at which increased amounts of free nicotine is delivered to the user [4].

The initial interaction of nicotine with the human body occurs most often in the oral cavity, where it is expected to be most active and its exposure to be most intense [5].

In India, the number of newly diagnosed tobacco-related cancers is estimated at approximately 250,000 out of a total of 700,000–900,000 new cancer cases diagnosed each year [6]. Thus, tobacco has been described as a major risk factor for oral cancer.

The effects of interaction of smokeless tobacco with the normal, indigenous microflora (especially in the oro-pharynx) are unknown. Little has been reported on the ability of nicotine to support or suppress the growth of micro-organisms [5]. This study was therefore, carried out to elicit the anti-microbial property of smokeless tobacco against *S. mutans* and to study the growth inhibition efficacy of three forms of smokeless tobacco extracts namely, raw smokeless tobacco, mishri and khaini.

## 2. MATERIALS AND METHODS

### 2.1 Saliva Collection

Five healthy individuals were selected for saliva collection according to the following critera:

a. Without any history of tobacco consumption in any form;
b. Zero DMFT index;
c. Individuals without history of medical illness.

Approximately 22 ml of un-stimulated whole saliva was collected from each subject in the morning half an hour after tooth brushing and the samples were stored at 4°C prior to processing.

## 2.2 Preparation of Extract

Smokeless tobacco in three different forms was used:

Group A: Raw tobacco leaves;
Group B: Mishri (Roasted powdered form of tobacco made by baking tobacco on a hot metal plate till it turns uniformly black [6]);
Group C: Khaini (A mixture of sun dried tobacco and slaked lime [6]).

Saliva and tobacco samples were autoclaved prior to the extract preparation. Two different extracts were prepared using saliva or ringer's lactate for each of the three different types of smokeless tobacco. Tobacco samples (7.5 g of each) were mixed with 7.5 ml of saliva or ringer lactate solution and incubated at 37°C for 2 hours while stirring intermittently during this period. After incubation, the mixture was centrifuged at 10,000 rpm for 5 min and the supernatants obtained were used as extracts.

## 2.3 Antimicrobial Sensitivity Assay

Brain Heart Infusion (BHI) culture plates were used for the study. Each culture plate had three wells. Two wells contained the 2 different tobacco extracts and the third well contained ciprofloxacin which was used as a positive control.

Similar process was carried out for all three forms of smokeless tobacco and all the 5 saliva samples, so we obtained total fifteen culture plates.

The culture plates were inoculated with the reference strain *S. mutans* ATCC 25175 and were incubated at 37°C for 24 hours. The effect of tobacco extracts on *S. mutans* was determined by the presence of a translucent halo around the wells, indicating inhibition of bacteria growth. The halo diameter of inhibition zones were measured (mm). These inhibition zones were later compared among the groups and with control (ciprofloxacin).

## 3. RESULTS AND DISCUSSION

Tobacco has been called the "Holy Herb" and "God's remedy" since ages and has been considered to have potential therapeutic properties [7]. Tobacco leaves are rich in alkaloids and polyphenols, mainly chlorogenic acid and rutin which possess good antimicrobial activity [4,8,9]. Tobacco is a known carcinogen and its use is the single greatest avoidable risk factor for cancer mortality worldwide. Tobacco predisposes to oral, esophageal and pancreatic cancer. Though the antimicrobial properties of tobacco are known since ages, literature search revealed very few studies on action of tobacco against *S. mutans*. So we attempted a study to shed light on the lesser known antimicrobial property of three forms of smokeless tobacco against *S mutans*.

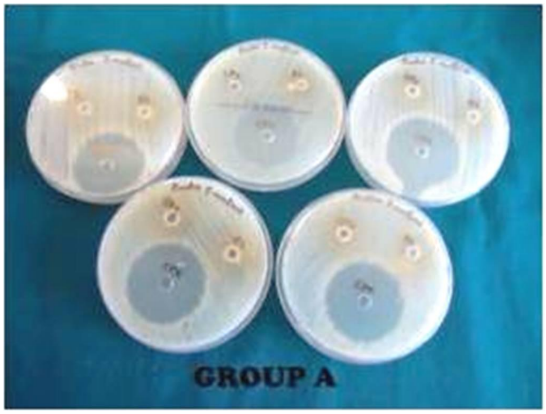


**Fig. 1. *S. mutans* growth inhibition zones for raw tobacco leaves extracts on BHI agar plates**

Above Fig. 1 shows the zones of growth inhibition for group A obtained after incubation at 37°C for 24 hours and Figs. 2 and 3 show the zones of inhibition for group B and C respectively.

The mean of zones of bacterial growth inhibition for khaini were higher than the other groups. After comparing the results obtained for all the three forms of smokeless tobacco, we found that khaini, a combination of sundried tobacco & slaked lime, showed comparatively larger inhibition zones among the three groups. (Table 1) The higher antimicrobial activity of khaini may be due to the added effect of lime in

reducing the bacterial count [10]. Raw tobacco leaves also showed positive results, thus, displaying the antimicrobial activity. Mishri (roasted tobacco) did not show antimicrobial property against *S. mutans*. This may be due to the destruction of the antimicrobial efficacy of the tobacco after the application of excessive heat, which is used to prepare mishri.

Our results are comparable with the results of the study done by Tandon et al. [5] wherein only raw tobacco was used. Though the mean of zones of bacterial growth inhibition for khaini were higher than the other groups, the results of comparison within groups were not statistically significant (Table 2).

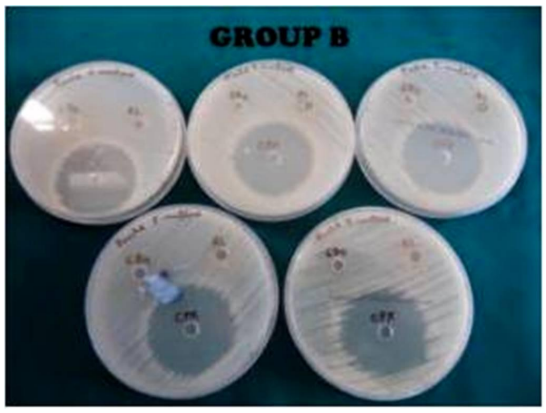


**Fig. 2. *S. mutans* growth inhibition zones for mishri extracts on BHI agar plates**

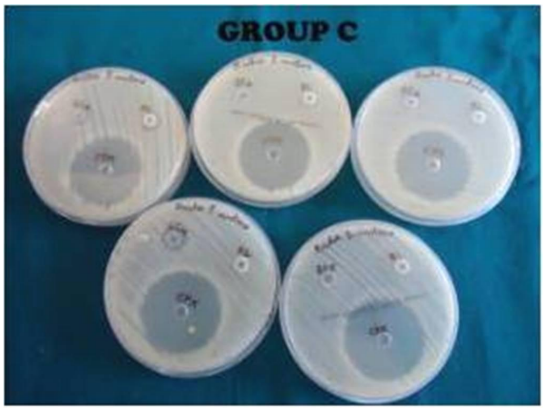


**Fig. 3. *S. mutans* growth inhibition zones for khaini extracts on BHI agar plates**

**Table 1. Values of halo diameter of growth inhibition zones for groups A, B and C using saliva & ringer's lactate solution**

| Group A | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Mean |
|---|---|---|---|---|---|---|
| Saliva | R* | R* | 11 mm | 10 mm | 10 mm | 6.2 |
| RL | R* | R* | 12 mm | 11 mm | 13 mm | 7.2 |
| CPX | 35 mm | 35 mm | 40 mm | 40 mm | 40 mm | 38 |
| **Group B** | **Sample 1** | **Sample 2** | **Sample 3** | **Sample 4** | **Sample 5** | **Mean** |
| Saliva | R* | R* | R* | R* | R* | 0 |
| RL | R* | R* | 8 mm | R* | R* | 0 |
| CPX | 40 mm | 40 mm | 40 mm | 40 mm | 40 mm | 40 |
| **Group C** | **Sample 1** | **Sample 2** | **Sample 3** | **Sample 4** | **Sample 5** | **Mean** |
| Saliva | 12 mm | 10 mm | 10 mm | 16 mm | R* | 9.6 |
| RL | 14 mm | R* | 11 mm | R* | R* | 5 |
| CPX | 40 mm | 40 mm | 40 mm | 40 mm | 40 mm | 40 |

*R*-resistant, RL-ringer's lactate, CPX- ciprofloxacin*

**Table 2. Statistical analysis by using ANOVA test**

| Source of variation | SS | | Df | | MS | | F | | P-value | | F crit | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S+T* | RL+T* | S+T | RL+T | S+T | RL+T | S+T | RL+T | S+T | RL+T | S+T | RL+T |
| Between Groups | 236.93 | 136.13 | 4 | 2 | 59.23 | 68.06 | 2.21 | 2.22 | 0.140 | 0.150 | 3.47 | 3.88 |
| Within Groups | 268 | 366.8 | 10 | 12 | 26.8 | 30.56 | | | | | | |
| Total | 504.93 | 502.93 | 14 | 14 | | | | | | | | |

*S + T*- saliva + tobacco extracts, RL + T*- ringer's lactate solution + tobacco extracts*
**P=0.140 for S + T and P=0.150 for RL + T*

Literature search reveals very few studies in this field which necessitates extension of this study using larger sample size. To use smokeless tobacco for anti- *S. mutans* activity, an attempt should be made to examine the tobacco leaves systematically for substances of high antimicrobial value.

Fractional distillation for specific antimicrobial substances in tobacco and combining them with appropriate agents would help to analyze commercial viability of smokeless tobacco as a source of antimicrobial agents. Further studies that would address issues such as adequate statistical power, standardization of extracts or purified compounds and quality control, would be of great value.

## 4. CONCLUSION

Amongst the three forms of tobacco analyzed, khaini has highest antimicrobial property on the growth of *S. mutans*, as compared to raw tobacco leaves and mishri. Tobacco leaves need to be systematically analyzed to be used as an anti-microbial agent.

.

## CONSENT

All authors declare that written informed consent was obtained from the patient for enrolling in the study.

## ETHICAL APPROVAL

The authors have obtained all necessary ethical approval from Institutional Ethical Committee**.**

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Palombo EA. Traditional medicinal plant extracts and natural products with activity against oral bacteria: Potential application in the prevention and treatment of oral diseases. Evid Based Complement Alternat Med. 2011;Article ID 680354.
2. Pereira CV, Pereira LJ, Gonçalves RB, Höfling JF. *In vitro* bacterial plaque suppression and recolonization by *S. Mutans* and *S.* Sobrinus. Braz J Microbiol. 2006;37:20–25.
3. Forssten SD, Björklund M, Ouwehand AC. *Streptococcus mutans*, Caries and Simulation Models. Nutrients. 2010;2:290-298. DOI:10.3390/nu2030290
4. Bhisey RA. Chemistry and toxicology of smokeless tobacco. Indian J Cancer. 2012;49:364-372.
5. Tandon A, Singh NN, Gadiputi S. Revealing anti-cariogenic efficacy of smokeless tobacco: A pilot study. J Oral Maxillo Fac Pathol. 2013;17(1):57–60.
6. Gupta PC, Ray CS. Smokeless tobacco and health in India and South Asia. Respirology. 2003;8:419–431.
7. Charlton A. Medicinal uses of tobacco in history. J R Soc Med. 2004;97:292–296.
8. Wang H, Zhao M, Yang B, Jiang Y, Rao G. Identification of polyphenols in tobacco leaf and their antioxidant and antimicrobial activities. Food Chemistry. 2008;107: 1399–1406.
9. Perumal SR, GopalaKrishnakone P. Therapeutic potential of plants as anti-microbials for drug discovery. Evid Based Complement Alternat Med. 2010;7:283–294.
10. Gleeson D. Evaluation of hydrated lime as a cubicle bedding material on the microbial count on teat skin and new intra mammary infection. Irish Journal of Agricultural and Food Research. 2013; 52:159–171.



*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*http://sciencedomain.org/review-history/10130*