ORIGINAL ARTICLE

Revealing anti-cariogenic efficacy of smokeless tobacco: A pilot study

Ankita Tandon, Narendra Nath Singh[1], Gadiputi Sreedhar[1]
Departments of Oral Pathology and Microbiology, Faculty of Dental Sciences, Chatrapati Shahuji Maharaj Medical University, Lucknow, [1]Oral Pathology, Kothiwal Dental College and Research Centre, Moradabad, Uttar Pradesh, India

Address for correspondence:
Dr. Ankita Tandon,
Department of Oral Pathology and Microbiology, Faculty of Dental Sciences, Chatrapati Shahuji Maharaj Medical University, Lucknow, Uttar Pradesh, India.
E-mail: tandon_ankita7@rediffmail.com

ABSTRACT

Background: The tobacco plant, *Nicotiana tabacum*, has been responsible for more deaths than any other herb. However, the literature has also been endowed with its use as "holy herb" since the pre-Columbian era. Used for treating pain, poisonous bites, ulcers, nasal polyps, and basal cell carcinoma; it also acts as an important ingredient of commercially available toothpastes; and even used as tobacco vaccines against *Streptococcus* species as highlighted in the literature. **Aims and Objectives:** (1) To elicit the anti-microbial property of tobacco against *Streptococcus mutans*, if any, in raw smokeless tobacco. (2) To study the relationship of duration and growth inhibition efficacy of smokeless tobacco. **Materials and Methods:** Extracts were prepared by centrifugation of mixed raw smokeless tobacco with Ringer's lactate solution and with saliva. The extracts were placed in wells prepared on Mitis salivarius culture plate and incubated at 37°C for 24 h after 0 h, 1 h, and 2 h of extract preparation. The inhibition zones were measured on the underside of plate using the vernier calipers. **Results:** Smokeless tobacco has a statistically significant zone of inhibition, which proves its anti-microbial activity against *S. mutans*. However, the mean zones of inhibition were greater for Ringer's lactate and tobacco group as compared to test samples (saliva and tobacco) with subsequent reduction of inhibition zones with an increase in duration. **Conclusion:** The anti-microbial property of extensive tobacco resources can be utilized from their extracts in order to balance the deterioration it had caused to mankind.

Key words: **Anti-cariogenecity, *Streptococcus mutans,* tobacco**

INTRODUCTION

Oral diseases are major health problems with dental caries and periodontal diseases among the most important preventable global infectious diseases. Oral health influences the general quality of life, and poor oral health is linked to chronic conditions and systemic diseases. The association between oral diseases and the oral microbiota is also well established. The development of dental caries involves acidogenic and aciduric gram-positive bacteria (*mutans streptococci*, *Lactobacilli*, and *Actinomycetes*), which metabolize sucrose to organic acids (mainly lactic acid) that dissolve the calcium phosphate in teeth, causing decalcification and eventual dental decay.[1] Streptococci of the *mutans* group are closely associated with dental caries, mainly those involving smooth surfaces. Production of acid and extracellular polysaccharides due to hydrolysis of sucrose facilitates their adhesion to tooth surfaces.[2]

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/0973-029X.110727

Tobacco, however, is a very important economic crop.[3] When the use of *Nicotiana* by the indigenous populations in the new world was first observed by Columbus and the plant was brought to Europe, all herbs were considered to have potential therapeutic properties. Indeed, *Nicotiana* acquired a reputation as a panacea, to the extent of being called the "holy herb" and "God's remedy."[4] 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] Tobacco variously called petum, betum, cogioba, cohobba, quauhyetl, picietl, or yietl, appeared later in herbals or pharmacopoeias.[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. Its possible

effect on immunity (especially near mucosal surfaces) has been described, while the effects of its interaction with the normal, indigenous microflora (especially in the oropharynx) is unknown; yet relatively high salivary concentrations (70-1,560 µg/ml) of nicotine are achievable for those using tobacco-based products. Along these lines, little has been reported on the ability of nicotine to support or suppress the growth of micro-organisms, and such studies have provided only inconsistent results[5] with numerous conflicting reports on the effect of local smokeless tobacco products on bacterial growth. This preliminary study was therefore, carried out to elicit the anti-microbial property of smokeless tobacco against *Streptococcus mutans*, if any, and to study the relationship of duration and growth inhibition efficacy of smokeless tobacco.

MATERIALS AND METHODS

An extract of smokeless tobacco was prepared by placing 7.5 mg of raw smokeless tobacco [Figure 1] in 7.5 ml Ringer lactate solution, and 7.5 ml of saliva from subjects with no tobacco habits and zero DMFT at 37°C for 2 h [Figure 2]. The mixture was stirred intermittently. After 2 h, the mixture was centrifuged beyond 2,500 rpm for 5 min and the supernatants obtained were used as extracts [Figure 3]. Mitis salivarius culture plates were prepared, and wells were made. The extracts were placed in the wells at 0 h, 1 h, and 2 h of extract preparation, respectively. The zones of inhibition were measured using vernier calipers on Mitis salivarius culture plate after incubation at 37°C for 24 h [Figure 4]. These inhibition zones were later compared among the groups and with control (anti-microbial disc). The growth on the culture plate was confirmed to be of *S. mutans* using Bio ID Strep kit (HiMedia) for sorbitol, mannitol, and catalase test, respectively.

RESULTS

There was a statistically significant difference among the groups. The mean zones of inhibition were of maximum size for anti-microbial disc and of minimum size for saliva tobacco at 2 h of extract preparation [Table 1]. The mean difference as regards to the zones of inhibition between and within the groups gave statistically significant results at 0.05 level

Figure 1: Raw smokeless tobacco

Figure 2: Mixtures of tobacco with Ringer's lactate solution and saliva

Figure 3: Extracts after centrifugation

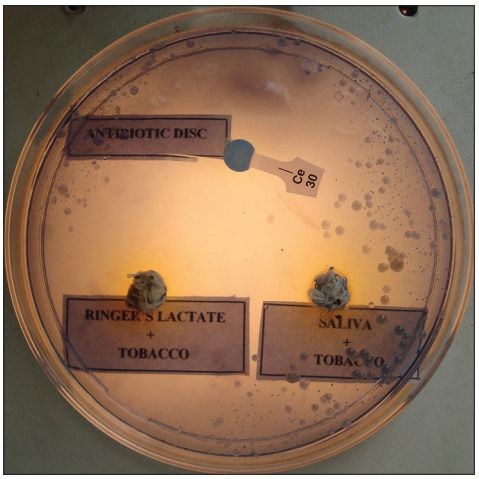

Figure 4: *Streptococcus mutans* colonies over Mitis salivarius culture plate growing beyond the zones of inhibition

Table 1: Comparison of mean zones of inhibition under different treatment conditions

Treatment conditions	*N*	Mean	Standard deviation	Standard error	95% confidence interval for mean		Minimum	Maximum
Anti-microbial disc	5	20.8000	0.83666	0.37417	19.7611	21.8389	20.00	22.00
Ringer lactate tobacco	5	18.0000	0.70711	0.31623	17.1220	18.8780	17.00	19.00
Saliva tobacco 0 h	5	8.4000	0.89443	0.40000	7.2894	9.5106	7.00	9.00
Saliva tobacco 1 h	5	7.3000	0.57009	0.25495	6.5921	8.0079	6.50	8.00
Saliva tobacco 2 h	5	5.9000	0.22361	0.10000	5.6224	6.1776	5.50	6.00
Total	25	12.0800	6.25113	1.25023	9.4997	14.6603	5.50	22.00

using the ANOVA test [Table 2]. Multiple comparisons also revealed a statistically significant difference among groups except for saliva with tobacco at 0 h versus saliva with tobacco at 1 h [Table 3].

Table 2: ANOVA test results

Comparison	Sum of squares	Degrees of freedom	Mean square	F variable	Sig.
Between groups	928.340	4	232.085	488.600	0.000
Within groups	9.500	20	0.475		
Total	937.840	24			

DISCUSSION

The tobacco plant, *Nicotiana* has probably been responsible for more deaths than any other herb. Undoubtedly, tobacco is the most important avoidable cause of premature death and disease in the world. Tobacco leaves and the smoke generated when they are burned contain over 4,000 chemicals, the best known of which is nicotine, first isolated from tobacco leaves in 1828 by Posselt and Reimann.[4]

Over 20% of tobacco resources are discarded as processing waste, which pollute the environment and cause a big waste. In fact, the discarded tobacco leaves are economically valuable because of abundant bioactive compounds in them. Therefore, it is important to investigate and utilize the resource of tobacco leaf.[3]

Interference with *S. mutans* ability to colonize teeth surfaces is an important strategy of dental caries prevention. In addition to being a vaccination target, inhibition of Glucosyl transferases GTFs and sucrose-dependent *S. mutans* colonization has been a subject of many *in vitro* studies in which different agents, including monoclonal and polyclonal antibodies, plant extracts, natural substances, andchemical reagents, were shown to possess such inhibitory properties. The active componentspresent in plant extracts are tannins and other polyphenols.[6] However, the major classes of compounds identified in tobacco include aliphatic and aromatic hydrocarbons, aldehydes, ketones, alcohols, phenols, amines, amides, alkaloids, metals, and radioelements. Tobacco-specific nitrosamines are formed from alkaloids during the processing of tobacco leaves.[7]

Smokeless tobacco in our study had statistically significant zones of inhibition which proves their anti-microbial activity against *S. mutans*. However, the mean zones of inhibition were greater for Ringer's lactate and tobacco as compared to test samples (saliva and tobacco). There was a subsequent reduction of inhibition zones with an increase in duration suggesting the rapid breakdown of the anti-microbial substances like polyphenols (chlorogenic acid and rutin) in tobacco[3] probably due to the activity of lytic enzymes. Although tannins, polyphenols, oils, and others have been identified as the effective component for the bacterial damaging or killing action in the *in vitro* system, many of these compounds failed in human clinical trials to determine their therapeutic effectiveness.[8] Therefore, *in vitro* observations from this study may not necessarily be obtained *in vivo* as such effect can be counteracted by the natural immune defense system.[7]

Contrary to this, Keen and Johnson reported a biphasic dose-dependent effect of nicotine on the growth of cariogenic *S. mutans* and suggested that the concentration of nicotine (10^{-3} M) reported within the saliva of smokeless tobacco users could actually stimulate growth of *S. mutans* and possibly place the user at risk for the dental caries. It is commonly known that loose-leaf tobacco contain sweeteners such as molasses or sugar, whereas, moist snuff contains few sweeteners. The sugar contents of tobacco products have been shown to vary from one form-to- another form, brand-to-brand, and region-to-region.[9] Studies published have suggested that chewing tobacco products contain very high levels of fermentable sugars (30-40% by weight). The sugars in these products are believed to contain glucose, fructose, sucrose, maltose, and isomaltose, which can increase the *in vitro* growth of the cariogenic bacteria, *S. mutans*.[10] Hence, raw smokeless tobacco was employed in the study in contrast to the smokeless tobacco products available in the market in order to avoid any bias due to the reported evidence of added sugar contents.

CONCLUSION

Tobacco has a strong anti-cariogenic effect. Despite of this fact, the use of smokeless tobacco product is not advised as an anti-caries measure in any raw form due to the known carcinogenic potential of tobacco. The findings of the aforementioned study are preliminary and hence, more

Table 3: Multiple comparisons

(I) Groups	(J) Groups	Mean difference (I-J)	Standard error	Sig.	95% confidence interval	
					Lower	Upper
Anti-microbial disc	Ringer lactate tobacco	2.80000*	0.43589	0.000	1.4957	4.1043
	Saliva tobacco 0 h	12.40000*	0.43589	0.000	11.0957	13.7043
	Saliva tobacco 1 h	13.50000*	0.43589	0.000	12.1957	14.8043
	Saliva tobacco 2 h	14.90000*	0.43589	0.000	13.5957	16.2043
Ringer lactate tobacco	Anti-microbial disc	−2.80000*	0.43589	0.000	−4.1043	−1.4957
	Saliva tobacco 0 h	9.60000*	0.43589	0.000	8.2957	10.9043
	Saliva tobacco 1 h	10.70000*	0.43589	0.000	9.3957	12.0043
	Saliva tobacco 2 h	12.10000*	0.43589	0.000	10.7957	13.4043
Saliva tobacco 0 h	Anti-microbial disc	−12.40000*	0.43589	0.000	−13.7043	−11.0957
	Ringer lactate tobacco	−9.60000*	0.43589	0.000	−10.9043	−8.2957
	Saliva tobacco 1 h	1.10000	0.43589	0.125	−0.2043	2.4043
	Saliva tobacco 2 h	2.50000*	0.43589	0.000	1.1957	3.8043
Saliva tobacco 1 h	Anti-microbial disc	−13.50000*	0.43589	0.000	−14.8043	−12.1957
	Ringer lactate tobacco	−10.70000*	0.43589	0.000	−12.0043	−9.3957
	Saliva tobacco 0 h	−1.10000	0.43589	0.125	−2.4043	0.2043
	Saliva tobacco 2 h	1.40000*	0.43589	0.032	0.0957	2.7043
Saliva tobacco 2 h	Anti-microbial disc	−14.90000*	0.43589	0.000	−16.2043	−13.5957
	Ringer lactate tobacco	−12.10000*	0.43589	0.000	−13.4043	−10.7957
	Saliva tobacco 0 h	−2.50000*	0.43589	0.000	−3.8043	−1.1957
	Saliva tobacco 1 h	−1.40000*	0.43589	0.032	−2.7043	−0.0957

*The mean difference is significant at 0.05 level

samples are required to validate the results. Furthermore, to use smokeless tobacco as an anti-caries measure, an attempt should be made to examine the tobacco leaves systematically for substances of high therapeutic value. Fractional distillation for specific anti-cariogenic substances in the tobacco leaves would therefore, help to analyze commercial viability of smokeless tobacco product as an anti-cariogenic measure.

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;2011:680354.
2. Pereira1 CV, Pereira LJ, Gonçalves RB, Höfling JF. *In vitro* bacterial plaque suppression and recolonization by *S. Mutans* and *S. Sobrinus*. Braz J Microbiol 2006;37:20-5.
3. Wang H, Zhao M, Yang B, Jiang Y, Rao G. Identification of polyphenols in tobacco leaf and their antioxidant and antimicrobial activities. Food Chem 2008;107:1399-406.
4. Charlton A. Medicinal uses of tobacco in history. J R Soc Med 2004;97:292-6.
5. Pavia CS, Pierre A, Nowakowski J. Antimicrobial activity of nicotine against a spectrum of bacterial and fungal pathogens. J Med Microbiol 2000;49:675-6.
6. Al-Hebshi NN, Nielsen O, Skaug N. *In vitro* effects of crude khat extracts on the growth, colonization, and glucosyl transferases of *Streptococcus mutans*. Acta Odontol Scand 2005;63:136-42.
7. Pershagen G. Smokeless tobacco. Br Med Bull 1996;52:50-7.
8. Perumal Samy R, Gopalakrishnakone P. Therapeutic potential of plants as anti-microbials for drug discovery. Evid Based Complement Alternat Med 2010;7:283-94.
9. Ayo-Yusuf OA, van Wyk C, van Wyk CW, de Wet I. Smokeless tobacco products on South African market do not inhibit oral bacterial flora: A pilot study. South Afr J Epidemiol Infect 2005;20:136-9.
10. Tomar SL, Winn DM. Chewing tobacco use and dental caries among U.S. men. J Am Dent Assoc 1999;130:1601-10.

How to cite this article: Tandon A, Singh NN, Sreedhar G. Revealing anti-cariogenic efficacy of smokeless tobacco: A pilot study. J Oral Maxillofac Pathol 2013;17:57-60.

Source of Support: Nil. **Conflict of Interest:** None declared.