ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Effects of refined extracts of smokeless tobacco on Cariogenic microorganisms: An in-vitro study

Sumeet Bhatt, GuruRaghavendran Rajesh, Mithun BH Pai, Ashwini Rao, Ramya Shenoy

Department of Public Health Dentistry, Manipal College of Dental Sciences, Manipal University, Mangalore, Karnataka, India

Manuscript Info

Manuscript History:

Received: 12 February 2014
Final Accepted: 23 March 2014
Published Online: April 2014

Key words:
Smokeless tobacco, Dental caries, Cariogenic microorganisms.

Corresponding Author

GuruRaghavendran Rajesh

Abstract

BACKGROUND & OBJECTIVE: This study attempts to investigate the effect of smokeless tobacco on the growth of cariogenic microorganisms of the genus Lactobacillus, Actinomyces and Candida.

METHODS: One percent of nutrient broth/cultures of Lactobacillus, Actinomyces and Candida were prepared containing 10% concentration of smokeless tobacco extract.Inoculated broths/cultures were suspended and their optical densities were measured by spectrophotometer as a guide to microbial growth at 24 and 48 hours. The mean for each test microorganism was calculated. Broth/culture without extract was used as control.

FINDINGS: The turbidometric method as a guide to growth of cariogenic microorganisms showed that there were significant differences between microbial growths of all the three microorganisms among control group and various forms of smokeless tobacco used ($p<0.05$). The results were statistically significant at 24 and 48 hours.

CONCLUSIONS: Smokeless tobacco extracts showed anti-microbial activity against Candida, Actinomyces and Lactobacilli species suggesting an anti-cariogenic effect. Further research into tobacco should be carried out to explore the anti-cariogenic components with high therapeutical value.

Introduction:

The smokeless form of tobacco has been in use in many countries for centuries. Use of these products has been a common practice in India and several other Asian countries, as well as in parts of Africa. However, during the past decades, an increase in the use of smokeless tobacco has been observed in the USA and some northern European countries, especially by young people (Boffettaet al., 2008).

Use of smokeless tobacco is an established risk factor for oral cancer. Risks of oesophageal cancer and pancreatic cancer due to smokeless tobacco use have also increased (Boffettaet al., 2008). Apart from being associated with cancers of oro-pharyngeal cancers, tobacco use has also been claimed to be associated with dental caries (Rooban et al., 2011), but scientific evidence supporting this claim is very limited.

Various studies have shown the effects of tobacco on the development of dental caries. Rooban et al, in a study done on Indian population, concluded that use of tobacco in any form seems to increase the risk for dental caries considerably (Rooban et al., 2011). This may be due to the changes in oral micro-flora owing to tobacco use and alcohol which could result in the initiation and progression of dental caries.Vellapally et al in a study done on Indian population found that DMFT value of tobacco chewers, regular smokers and ex-smokers is higher when compared to non-tobacco users, thus establishing the fact that chewing tobacco and smoking can present significant risk factors for dental caries (Vellapally et al., 2008).A review has concluded that oral use of smokeless tobacco is a positive contributing factor for a higher dental caries incidence especially tobacco chewing. One of the main reasons

for this type of association between smokeless tobacco and dental caries was cited as the presence of high quantity of various types of sugars and sweeteners added during the commercial manufacturing of smokeless tobacco products (Vellapally et al., 2007).Similar findings were reported by Tomar et al among adult males in United States where chewing tobacco was reported as a risk factor for development of root surface caries and coronal caries. One of the reasons cited for this association was the high sugar content in smokeless tobacco (Tomar et al., 1999).

The types of sweeteners and sugars commonly found in smokeless tobacco are fructose, glucose, sucrose, maltose, and isomaltose (Hsu et al., 2007).The addition of sweeteners is supposed to neutralize the bitter taste of tobacco (Talhout et al., 2006).The presence of these high levels of fermentable sugars in the smokeless tobacco can stimulate the growth of cariogenic microorganisms by providing a suitable environment conducive to their growth (Going et al., 1980; Tomar et al., 1999).Some in-vitro studies have supportedthis theory wherein the growth of cariogenic microorganisms such as Streptococcus mutans (Lindemeyer et al., 1981; Hiregoudar et al., 2010; Falker et al., 1987) and Streptococcus sanguis (Falker et al., 1987; Tomar et al.,1999) was stimulated in the presence of smokeless tobacco.

Most of the previous studies (Offenbacher and Weathers, 1985; Keene and Johnson, 1999; (Nagarajappa and Prasad, 2010;Huang et al., 2012; Tandonet al., 2013) have been done on the cariogenic streptococci suggesting that smokeless tobacco may be implicated in the initiation of dental caries. However, the evidence on the effect of smokeless tobacco on other microorganisms which are responsible for progression of dental caries is scarce. These include microorganisms such as Lactobacilli and Candida. The data on effects of smokeless tobacco on microorganisms involved in root caries such as Actinomyces is also lacking. Therefore, it would be of important significance to investigate the effect of smokeless tobacco on the growth of cariogenic microorganisms of the genus Lactobacillus, Actinomyces and Candida.

Therefore, the present study was conducted with the objective of assessing the effects of smokeless tobacco extracts on the growth of species of Lactobacilli, Actinomyces, and Candida albicans.

Methodology:

The study was conducted in Department of Public Health Dentistry, Manipal College of Dental Sciences, Mangalore, Department of Pharmacology, Kasturba Medical College, Mangalore and Department of Microbiology, Maratha Mandal's NGH Institute of Dental Sciences & Research Centre, Belgaum.

The present study was an in-vitro experimental study and different forms of smokeless tobacco were included in the study. Local popular brands of tobacco such as gutkha, khaini, and unprocessed tobacco were obtained from the local market of Mangalore city, Karnataka. A total of 100 grams of smokeless tobacco was put in a dialysis bag in a flask containing 100 ml of distilled water and suspended for 48 hours at room temperature. After 48 hours, suspension was centrifuged for 20 minutes at 2500 rpm. Supernatant was filtered, sterilized and stored at a temperature of 4 degree Celsius (Falker et al., 1987).

Microorganisms include in the present study were Lactobacilli, Actinomyces and Candida albicans Microorganisms were be incubated for 24-48 hours at 37 degrees Celsius in the Department of Microbiology, Maratha Mandal's NGH Institute of Dental Sciences & Research Centre, Belgaum.

Turbidity measurement by spectrophotometer:

1% of nutrient broth/culture was prepared containing 10% concentration of smokeless tobacco extract. After autoclaving, broth/culture and extracts were inoculated with microbial inoculums adjusted to 10^6 CFU/ml and incubated. Inoculated broths/cultures were suspended and their optical densitieswere measured by spectrophotometer as a guide to microbial growth at 24 and 48 hours. Experiments were performed in triplicates for each extract and the mean for each test microorganism was calculated. Broth/culture without extract was used as control (Mat Ludin and Md Radzi, 2001).

Statistical Analysis

One-way ANOVA followed by post-hoc Tukey test was performed to compare the means of the tobacco extract-treated and the untreated groups. The level of significance was 0.05 for statistical hypothesis testing.

Results:

The turbidometric method as a guide to growth of cariogenic microorganisms showed that there were significant differences between microbial growths of all the three microorganisms among control group and various forms of smokeless tobacco used ($p<0.05$). The results were statistically significant at 24 and 48 hours.

CANDIDA SPECIES (Fig. 1)

Post hoc analysis showed that at 24 hours, the mean optical density of microorganisms was significantly higher in control culture as compared to microbial inocculum grown in various forms of smokeless tobacco ($p<0.05$). However, no significant differences were observed among various tobacco forms.

At 48 hours, mean optical density of control culture was significantly higher than all three forms of smokeless tobacco ($p<0.05$). Among various tobacco forms, the mean optical density of cultures grown with extracts of gutkha was significantly higher than unprocessed tobacco and khaini ($p<0.05$). No significant differences were observed between unprocessed tobacco and khaini.

ACTINOMYCES (Fig. 2)

Significant differences were noted between control and all the forms of smokeless tobacco at both 24 and 48 hours ($p<0.05$). Significant differences were also observed among mean optical densities of various tobacco forms ($p<0.05$). Maximum growth was observed with khaini followed by unprocessed tobacco and gutkha. At 48 hours, maximum stimulatory effect was observed with unprocessed tobacco followed by khaini and gutkha.

LACTOBACILLI (Fig. 3)

Significant differences were noted between control and all three forms of smokeless tobacco at both 24 and 48 hours ($p<0.05$). Mean optical densities of various tobacco products also showed significant differences at both time periods. At 24 hours, maximum mean optical density was observed in cultures grown in gutkha followed by khaini and unprocessed tobacco. At 48 hours, maximum optical density was exhibited by khaini followed by unprocessed tobacco and gutkha.

Figures:

Fig. 1 Optical densities of Candida species at 24 and 48 hours.

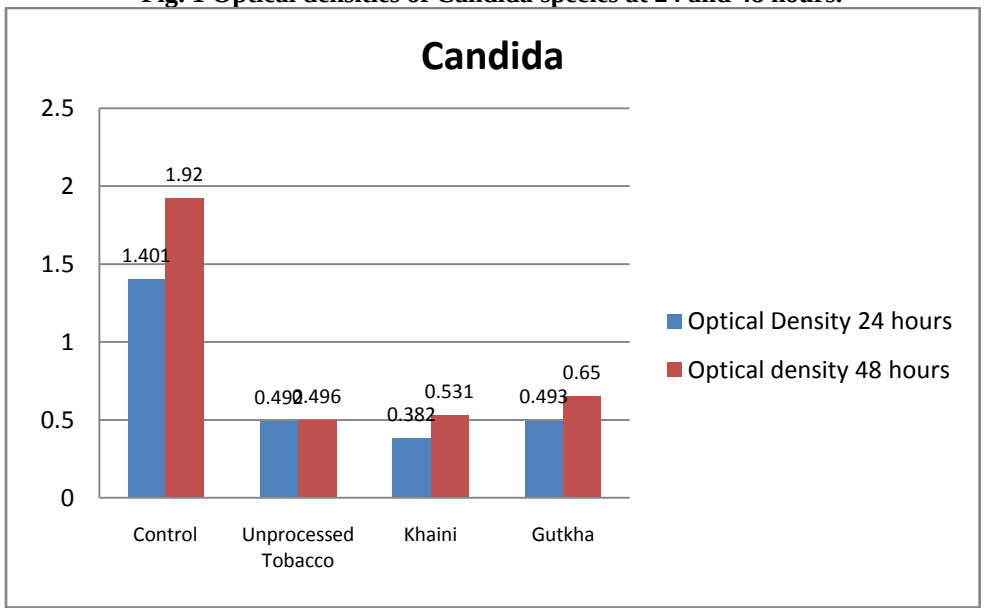


Fig. 2 Optical densities of Actinomyces species at 24 and 48 hours.

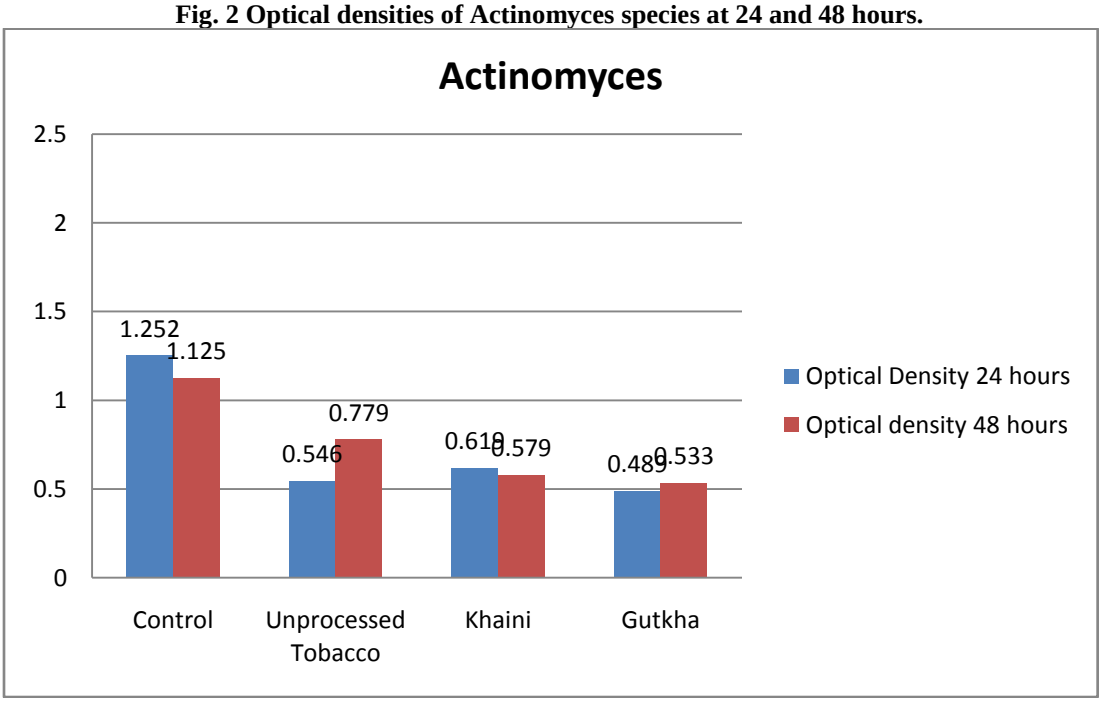
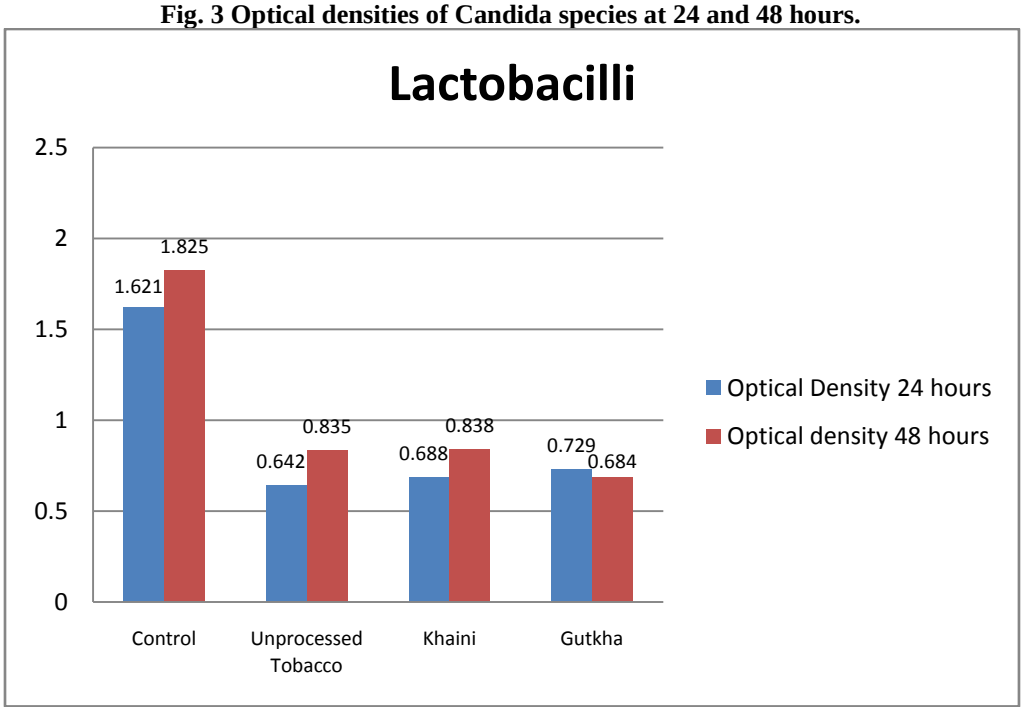


Fig. 3 Optical densities of Candida species at 24 and 48 hours.



Discussion:

To our knowledge, this is one of the first studies to explore the effects of various forms of smokeless tobacco on the growth of candida, actinomyces and lactobacillus species. Previous studies in literature have focussed on effect of tobacco on Streptococcus mutans, thus establishing its role in initiation of caries. The present study attempts to explore the effects of various forms of smokeless tobacco on cariogenic microorganisms which are implicated in the progression of dental caries.

The plant of Nicotiana has been implicated in a large number of deaths because of various health problems attributed to the use of tobacco. Tobacco can be considered as one of the most important preventable causes of premature morbidity and mortality in the world. Tobacco products contain over 4,000 chemicals, most of which are harmful for health (Charlton et al., 2004).

In this study, smokeless forms of tobacco showed significant anti-microbial activity against all the three microorganisms. This finding was evident from the fact that optical densities of cultures grown at a 10% concentration of extracts of smokeless tobacco were significantly lesser than control cultures. This implies that the presence of smokeless tobacco extracts inhibited the growth of these microorganisms.

Previous studies done to assess the effects of tobacco on cariogenic microorganisms have reached differing results. Huang et al, in an in-vitro study, found an association between nicotine and growth of Streptococcus mutans (Huang et al., 2012). They found that nicotine enhances S. mutans biofilm formation and biofilm metabolic activity, thus suggesting that smoking canincrease the development of caries by fostering increased formation of S. mutansbiofilm on tooth surfaces. Similar findings were reported by Falker et al who found that extracts of smokeless tobacco can serve as a growth extract for various species of cariogenic streptococci, suggesting a stimulatory role of smokeless tobacco on cariogenic microorganisms (Falker et al., 1987). These findings tend to suggest that tobacco is a contributory factor in the development and initiation of dental caries.However, in an in-vitro study, Tandon et al observed that smokeless tobacco extract had a statistically significant zone of inhibition for S. mutans, suggesting its anti-microbial activity (Tandon et al., 2013).

An inherent limitation of the present study is its in-vitro design so the results cannot be generalized to the general population. However, the findings of this study are important in the context of dental caries pathology. Further studies involving human subjects with robust biochemical and microbiological testings can be done to establish the anti-cariogenic effects of smokeless tobacco. The component of smokeless tobacco responsible for such anti-cariogenic effect, if any, can be extracted and used for further caries research.

Conclusions:

Smokeless tobacco extracts were found to exhibit anti-microbial activity against three cariogenic microorganisms, namely; Candida, Actinomyces and Lactobacilli species. Despite the observed anti-cariogenic effect, the use of smokeless tobacco cannot be recommended as a caries preventive measure because of the addictive nature and established carcinogenic effect of tobacco.

The findings of the present study can serve as a ground work for future research in the field of dental caries. Tobacco should be studied in detail to find out the components with high therapeutical significance.

References:

1. Boffetta, P., Hecht, S., Gray, N., Gupta, P. and Straif, K. (2008): Smokeless tobacco and cancer. Lancet. Oncol., 9(7):667-75.
2. Charlton, A. (2004):Medicinal uses of tobacco in history. J. R. Soc. Med., 97:292–6.
3. Falkler, W.A. Jr, Zimmerman, M.L., Martin, S.A. and Hall, E.R. (1987):The effect of smokeless-tobacco extracts on the growth of oral bacteria of the genus Streptococcus.Arch. Oral. Biol., 32(3):221-3.
4. Going, R.E., Hsu, S.C., Pollack, R.L. and Haugh, L.D. (1980):Sugar and fluoride content of various forms of tobacco. J. Am. Dent. Assoc., 100(1):27-33.
5. Hiregoudar, M., Subramaniam, R., Mittal, S., Prashant, G.M. and Chandu, G.N. (2010):In vitro effect of smokeless tobacco extracts on the growth of Streptococcus mutans in relation to Nicotine and sugar content. J.Ind. Assoc. Public. Health. Dent., 16:174-178.
6. Hsu, S.C., Pollack, R.L., Hsu, A.F. and Going, R.E. (1980):Sugars present in tobacco extracts. J. Am. Dent. Assoc., 101(6):915-8.

7. Huang, R., Li, M. and Gregory, R.L. (2012):Effect of nicotine on growth and metabolism of Streptococcus mutans. Eur. J. Oral. Sci., 120(4): 319–325.

8. Keene, K. and Johnson, R.B. (1999):The effect of nicotine on growth of Streptococcus mutans. Miss. Dent. Assoc. J., 55(4):38-9.

9. Lindemeyer, R.G., Baum, R.H., Hsu, S.C. and Going, R.E. (1981):In vitro effect of tobacco on the growth of oral cariogenic streptococci. J. Am. Dent. Assoc., 103(5):719-22.

10. Mat Ludin, C.M. and Md Radzi J. (2001):The antimicrobial activity of different mouthwashes in Malaysia. Malays. J. Med. Sci., 8(2):14-8.
11. Nagarajappa, S. and Prasad, K.V.(2010):Oral microbiota, dental caries and periodontal status in smokeless tobacco chewers in Karnataka, India: a case-control study. Oral. Health. Prev. Dent., 8(3):211-9.

12. Offenbacher, S. and Weathers, D.R. (1985):Effects of smokeless tobacco on the periodontal, mucosal and caries status of adolescent males. J. Oral. Pathol., 14(2):169-81.
13. Rooban, T., Vidya, K., Joshua, E., Rao, A., Ranganathan, S., Rao, U.K. and Ranganathan, K. (2011):Tooth decay in alcohol and tobacco abusers. J. Oral. Maxillofac. Pathol., 15(1):14-21.
14. Talhout, R., Opperhuizen, A. and van Amsterdam, J.G. (2006):Sugars as tobacco ingredient: effects on mainstream smoke composition. Food. Chem. Toxicol., 44(11):1789-98.
15. Tandon, A., Singh, N.N. and Sreedhar, G.(2013):Revealing anti-cariogenic efficacy of smokeless tobacco: A pilot study.J.Oral. Maxillofac. Pathol., 17(1):57-60.
16. Tomar, S.L. and Winn, D.M. (1999):Chewing tobacco use and dental caries among U.S. men. J. Am. Dent. Assoc., 130(11):1601-10.
17. Vellappally, S., Fiala, Z., Smejkalová, J., Jacob, V. and Shriharsha, P. (2007):Influence of tobacco use in dental caries development. Cent. Eur. J. Public. Health., 15(3):116-21.
18. Vellappally, S., Jacob, V., Smejkalová, J., Shriharsha, P., Kumar, V. and Fiala, Z. (2008):Tobacco habits and oral health status in selected Indian population. Cent. Eur. J. Public. Health., 16(2):77-84.