Original Article

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Efficiency of Tulsi Mouthwash against *Streptococcus mutans* and Salivary pH: An In-vivo Study

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ABSTRACT

Introduction: Salivary pH and *Streptococcus mutans* (*S mutans*) are the two main factors in causing dental caries and therefore targeting these may become an effective way to combat this worldwide disease. Tulsi (*Ocimum sanctum*) is an Indian herb with antibacterial properties and is used to treat variety of illnesses. The present study was conducted to evaluate the effect of Tulsi mouthwash on salivary pH and salivary *S mutans*.

Methodology: A total of 40 patients in the age group of 20-45 years with decay-missing-filled teeth index score of 3-4 were included in the study and randomly divided into two groups. The study group was given 5 ml Tulsi mouthwash, to be used every day for ninety days. The control was given 5 ml saline for the same period. Salivary pH and *S mutans* were analysed on 14th and 90th day.

Results: Tulsi mouthwash was able to reduce the *S mutans* colony and also raised the salivary pH towards normal.

Conclusion: Tulsi mouthwash being herbal can be an effective and safe alternative to the conventional mode of treatment.

INTRODUCTION

Dental caries and periodontal diseases, the two arch criminals of oral cavity, are essentially caused by the microorganisms present in dental plaque.[1] Dental caries is a complex process in which multiple intrinsic and environmental factors influence and initiate the progression of disease. Two such parameters are salivary pH and bacteria.[2] The salivary pH may be considered as the master variable since it influences most chemical reactions occurring in the oral cavity, especially the equilibrium between calcium phosphate of the tooth and the surrounding liquid phase. Thus, control of salivary pH can greatly control the caries activity in patients.[3]

Streptococcus mutans has been identified as the causative organism for dental caries and it is uniformly agreed that caries cannot occur without them. Oral cavity is an excellent breeding place for these microorganisms to multiply. Elimination or reduction of such pathogenic bacteria is beneficial in controlling dental caries.[2] Mouthwash solutions are aimed at ensuring sufficient oral hygiene. Their ease of use, in addition to their significant ability to reduce dental plaque formation, has made mouthwashes a reasonable method to limit gingivitis and periodontitis.[4]

An ideal antibacterial mouth rinse must be effective, act rapidly against microorganisms, maintain activity at low concentration, possess substantivity, be usable without causing discomfort, and economical.[5] All such properties are known to be present in Tulsi. Tulsi, scientifically known as *Ocimum sanctum*, is one such herb that is bestowed with enormous antimicrobial activity and is used to treat a variety of illnesses such as diabetes mellitus, arthritis, bronchitis, skin diseases, and dental caries.[6] In addition, compared to other herbal medicines, Tulsi is abundantly available, easily accessible, economically feasible, culturally acceptable, and may possess minimal side-effects and hence can be recommended for long-term use.[7] Therefore, the present study was conducted to assess the efficiency of Tulsi mouthwash in lowering salivary pH and *S mutans*.

METHODS

A triple blinded randomized control trail was conducted among patients attending the OPD in a private Dental College and Hospital. The study was carried out in the departments of Oral Pathology and Microbiology following clearance from the ethical committee of the institute. A total of 40 patients were included in the study and were randomly divided into two groups of 20 patients each. Patients aged between 20-45 years with decayed, missing, and filled teeth (DMFT) score 3-4 were included in the study. Patients with history of antibiotic consumption in recent past were excluded from the study. Following the selection of patients, they were briefed about the study after which a written consent was obtained.

Preparation of Tulsi mouthwash

The test material was prepared with the help of commercially available fine powder of sun dried Tulsi leaves and the powder was macerated with 100% ethanol. The mixture was filtered with the help of Whatman filter paper and the filtrate was reduced at low temperature (less than 60°C) to obtain a solid residue of Tulsi.[6] The extract was diluted with dimethyl formamide, an inert solvent, to obtain the desired 4% concentration of extract.[6] This was followed by dispensing Tulsi mouthwash to subjects in study group with instructions to rinse their mouth with 5 ml of Tulsi mouthwash for approximately 30 seconds once a day following breakfast for a period of ninety days. In contrast, subjects in the control group were given de-ionized distilled water with instructions being the same.

The subjects were also instructed to report on 14th and 90th day of the study for further follow up. Estimation of salivary pH and *Streptococcus mutans* colony count was carried out on subjects of both the groups at baseline, 14th, and 90th day of the study and recorded.

Collection of saliva and estimation of salivary pH

The oral cavity was rinsed thoroughly with water and approximately 2 ml of unstimulated whole saliva was collected in a sterile container by asking the patient to expectorate gradually over a period of five minutes.[7]

Estimation of *Streptococcus mutans*

Media preparation: The media used was mitis salivarius bacitracin agar. 10 gm of mitis salivarius agar was suspended in a flask containing 100 ml of distilled water and mixed to dissolve completely. 10% sucrose solution was added to promote the growth of *Streptococcus mutans* followed by 1 mg of bacitracin.[8] Sterilization was done by autoclaving at 121°C for 15 minutes at 15 lbs. Then it was cooled to 55°C before 1 ml of sterile 1% potassium tellurite was added to increase the selectivity of media for streptococcus.[7] Streaking was done using an inoculation loop and then the plates were incubated under aerobic condition for 48 hours at 37°C for isolation of *Streptococcus mutans*.[7] Following incubation, the colonies of *Streptococcus mutans* were identified and confirmed with gram stained smear showing gram positive cocci in chain.[8]

RESULTS

In both the study and control groups, there was a rise in salivary pH from baseline to 14th day and 90th day. The rise in pH in both the groups was statistically significant

Table 1: Comparison of mean salivary pH at different time points between study and control group

Group	Time Period	Mean	'p' value		
			Baseline-14th day	Baseline-90th day	14th day –90th day
Study	Baseline	6.39	0.009*	0.0044*	0.002*
	14th day	6.48			
	90th day	6.62			
Control	Baseline	6.54	0.007*	0.008*	0.009*
	14th day	6.56			
	90th day	6.61			

*p≤ 0.05 Significant

Table 2: Comparison of mean *Streptococcus mutans* colony count between different time period in study and control group

Group	Time Period	Mean (cfu/ml)x10^4	'p' value		
			Baseline-14^{th} day	Baseline-90^{th} day	14^{th} day –90^{th} day
Study	Baseline	13.25	HS	HS	HS
	14^{th} day	12.4			
	90^{th} day	11.4			
Control	Baseline	13.1	HS	HS	HS
	14^{th} day	13.2			
	90^{th} day	14.4			

HS: Highly Significant ($p<0.001$)

(Table1). *S mutans* colony count decreased in the study group from baseline to 14^{th} day and 90^{th} day and this decrease was statistically significant. On the other hand, in the control group *S mutans* colony count increased from baseline to 14^{th} and 90^{th} day (Table 2). In addition, the differences in mean salivary *S mutans* colony count from baseline to 90^{th} day between the study and control group was found to be statistically significant ($p\leq 0.05$). The differences in mean were 1.85 ± 1.23 for study group and 1.13 ± 4.27 for control group.

DISCUSSION

Dental caries is a complex and dynamic process where a multitude of factors influence and initiate the progression of disease.[9] Inspite of the various available preventive measures, dental caries still persist to be a major problem in dentistry and should therefore receive significant attention. Tulsi is an Indian herb which is bestowed with enormous medicinal properties and is rightly called the "Queen of herbs". The chemical composition of Tulsi is highly complex, however, of the many active components that have been identified and extracted, the best known include eugenol (an essential oil), urosolic acid, carvacrol, and tannins, to name a few.[10] The nutritional and pharmacological properties of the whole herb in natural form, as it has been traditionally used, result from synergistic interaction of many phytochemicals, consequently, the overall effect of Tulsi cannot be fully duplicated with isolated compounds or extracts.[10] Literature review reveals that the anti-microbial property of Tulsi has been tested against a variety of microorganisms like *Candida albicans, Staphyloccus aureus,* and *Streptococcus mutans* to name a few.[6,11,12]

The increase in salivary pH observed in the study group could be due to the buffering capacity of Tulsi extract resulting from salivary stimulation due to its taste which increases the saliva's bicarbonate concentration and/or antibacterial activity of Tulsi against acid producing bacteria.[9,13,14] Willerhausen et al[15] in their study had observed a shift in the salivary pH values to the alkaline range in the herbal extract group. The possible explanation for the increase in pH in the control group could be that any mechanical stimulation in the form of mouth rinse can increase salivary flow rate thus leading to increase in salivary buffering capacity, as salivary stimulation leads to increase in the concentration of bicarbonate in the saliva.[13] The comparison of the mean salivary pH between study and control group at all study periods was found to be statistically insignificant ($p>0.05$).

The present study cannot attribute the anti-bacterial activity observed here to any particular component of Tulsi. However, the possible explanation for this effect can be the anti-bacterial agents present in Tulsi such as eugenol (l-hydroxy-2-methoxy-4-allylbenzene), urosolic acid, carvacrol, linalool, limatrol, and methyl carvicol.[9,16-19] The other possible explanation for the efficacy of Tulsi extract may be its wide variety of secondary metabolites such as flavonoids, terpenoids, alkaloids, and tannins which have been found in vitro to have anti-microbial properties.[13] These phytochemicals form high molecular weight complex with soluble proteins in saliva, increase bacteriolysis on tooth surface and in saliva, and interfere with bacterial adherence mechanisms on tooth surfaces.[2,8,20]

This change in count can be attributed to factors like maintenance of oral hygiene and change in dietary habits

such as shift towards cariogenic diet.[9] The significant difference observed between study and control group towards the end of study could be due to temporary intra oral deposits of tannins which are formed on regular rinsing with Tulsi extract. Tannins have large phenolic groups that provide them with unique binding properties causing them to bind to mucosal and tooth surfaces and this result in prolonged action of the extract.[13]

The present study indicates that Tulsi mouthwash reduced the salivary count of *Streptococcus mutans* and also increased salivary pH. The study thus implies that Tulsi mouthwash possess anti-bacterial activity against *Streptococcus mutans* as tested in vivo and may be used as an adjunct to prevent dental caries and to maintain good oral hygiene. In the present study, Tulsi mouth wash showed a definite reduction in the bacterial activity and an increase in pH resulting in marked anti cariogenic effect.

CONCLUSION

Use of Tulsi mouthwash demonstrated good anti-bacterial effect by reducing caries producing bacteria, *Streptococcus mutans* and reducing increased salivary pH suggests a positive role in reduction and prevention of dental caries. This preliminary in vivo study demonstrated the anti-bacterial and anti-cariogenic effect of Tulsi which indicated the use of Tulsi mouthwash as a meaningful and cost effective addition to mechanical oral hygiene method.

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