Research Article

Comparative Evaluation of the Effects of Black Tea Extract Mouthrinse and Chlorhexidine Mouthwash on Salivary Streptococcus Mutans Load

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ABSTRACT:

Background- Dental caries is one of the most frequent oral health problems. The present study shows the antibacterial effect of black tea extract on salivary *Sterptococcus Mutans* load.

Materials & Methods- The study was conducted on 125 individuals. The differences in the Colony Forming Units and count-scores of *S.mutans* were analyzed in salivary samples collected from individuals before and after administration of 2% black tea extract mouth-rinse and chlorhexidine mouthwash(CM).

Results- There was a statistical difference in mean salivary *S. mutans* colony count and mean count-score before and after administration of black tea extract mouth-rinse ($p = 0.0003$) and chlorhexidine mouthwash ($p = 0.0002$) respectively. Hence, it was found that there is no statistically significant difference in the fall of *S.mutans* load due to black tea mouth-rinse and chlorhexidine mouthwash.

Conclusions- A 2% black tea extract mouth-rinse significantly reduces salivary *S.mutans* load, irrespective of age and gender. Also, it is an effective natural anti-cariogenic agent with no known implicated side effects.

KEYWORDS: dental caries; oral health; streptococcus mutans; black tea extract mouth-rinse; chlorhexidine mouthwash.

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INTRODUCTION:

Dental caries is a public health problem throughout the world. In the western world, the prevalence of caries has declined, but 5-20% population still remain at high risk.[1] Many factors, both local like diet, tooth structure and anatomy, saliva, plaque, crevicular fluid, bacteria and systemic like age, gender, race, religion, culture, familial and genetic factors, socio-economic and nutritional status influence the likelihood of caries developing and its speed of progression, so that caries is truly a multifactorial disease.[2] The association of Streptococcus mutans (*S.mutans*) and dental caries was first reported by Clarke (1924). Since then, the experiments with gnotobiotic animals have revealed mutans streptococci to be the main etiological

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microorganisms in causation of dental caries.

The *S.mutan* is a facultatively anaerobic, gram-positive coccus. The mutans streptococci comprise of a group of seven species (mutans, sobrinus, cricetus, rattus, downeii, ferus and maccae). *S.mutans* and Streptococcus sobrinus are the predominant species isolated from human saliva and dental plaque.[3] These organisms are unique in their cariogenic potential. They are acidogenic and aciduric and once established, they can survive even in unfavourable conditions. Besides, the microorganisms in dental plaque degrade the dietary carbohydrates producing lactic acid leading to localized demineralization and the eventual formation of dental caries. *S.mutans* also encourage the accumulation and adherence of plaque biofilm by metabolizing sucrose into sticky glucan. It is now confirmed that *S. mutans* are the major bacteria responsible for the initiation of a carious lesion followed by Lactobacillus species which may be responsible for caries progression.[4]Thus, the obvious role of *S mutans* that was reflected all the way long in the causation of dental caries, justifies the need for its elimination or reduction in order to prevent occurrence of carious lesions.

Drug resistance and side effects encountered with the use of synthetic drugs has led to the surge for novel and safe alternatives. Since ancient times, plants have proven to be an archetypal source of medicine. One such plant of high medicinal use is the black tea (Camellia sinensis). The leaves of this plant are usually handpicked and based on the processing of the leaves three different types of tea are produced, namely green tea (non-fermented), Oolong tea (semi-fermented) and Black tea (fermented). Black tea being the fermented type possesses more raw nutrients and health effective compounds than the other two types.[5]Data available also enumerates some of the anticariogenic actions of certain components of black tea like bringing about remineralization of the dental hard tissues due to the release of calcium, phosphorus and fluoride ions.[6] The purpose of this study was to analyse the effect of black tea extract mouth-rinse,a natural measure with reduced side effect, in the prevention of dental caries.

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MATERIALS & METHODS:

This study was carried out with the approval of Institutional Ethical Committee and Bhabha College of Dental Sciences, Bhopal. The study was conducted in the Department of Oral Pathology & Microbiology on individuals in the age range of 21-40 years and a written informed consent for the procedure was obtained from them.

A total of 125 individuals in the age range of 21-40 years were randomly selected as per the inclusion and the exclusion criteria from those reporting to the Bhabha College of Dental Sciences, Bhopal. They were divided into three groups (a) administered Black tea extract mouth-rinse (BTEMR) in 50 subjects (b) administered Chlorhexidine mouthwash (CM) in 50 subjects and (c) administered distilled water in 25 subjects.

Inclusion Criteria-

Individuals with or without dental caries in the age group of 21-40 years.

Exclusion Criteria-

a) Completely or partially (more than 4 teeth missing) edentulous patients; b) patients who had received any antibiotic therapy in the last 14 days prior to the study; c) patients who underwent topical fluoride application in the last 3 days or mouth wash gargles within the last 12 hours prior to the study and d) patients with any known systemic disease.

BTEMR made by 2 gm of dried black tea leaves were boiled in 100 ml of tap water for 3 minutes in a stainless-steel utensil. The solution was allowed to cool for 10 minutes and then sieved into a disposable glass. This BTEMR was prepared fresh before use.

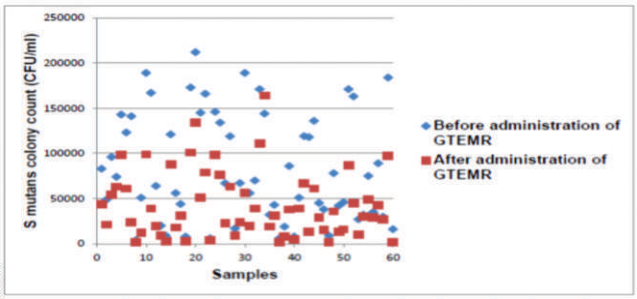
The individuals were briefly explained about the procedure prior to sample collection. They were instructed to maintain their normal oral prophylaxis and were instructed to avoid brushing or eating 1 hour prior to the saliva collection. Unstimulated saliva sample was collected from all the individuals participating in the study prior to and after the administration of BTEMR. Each individual of the study population was instructed to spit around 1-2 ml of saliva in a sterile wide mouth glass bottle. After having collected the first saliva sample, the individuals were instructed to gently rinse their mouth with 10-20 ml of the prepared BTEMR for 2 minutes and a second saliva sample was collected again after 30 minutes in another sterile glass bottle. The same procedure was followed for the other two groups, where instead of BTEMR, Chlorhexidine Mouthwash (CM) or distilled water were administered.

Culture media was prepared as follows:90 gm of Mitis Salivarius dehydrated agar was added to 1 litre of distilled water and boiled on a Bunsen burner to dissolve completely. To this 20 gm of sucrose per 100 ml was added. This solution was then sterilized by autoclaving for 15 minutes at 15 lb per square inch at 121^0C. The solution was allowed to cool to 50^0C.Potassium tellurite (0.1 mg/ml), bacitracin (0.2

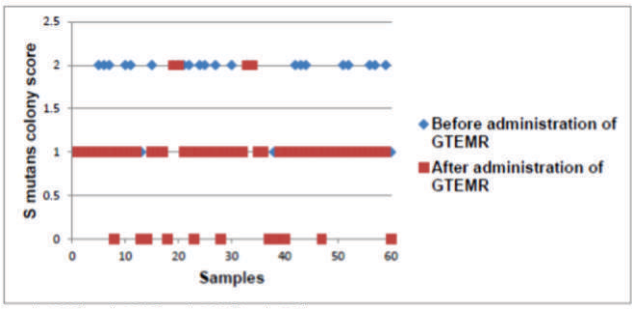
Table 1: Distribution and comparison of the salivary *S.mutans* colony count (CFU/ml) and the colony score in all the subjects before and after the administration of BTEMR.

		N	Mean	Standard Deviation	Difference of means	*p*-value
CFU/ml	Before	50	8.3700	5.9713		<0.0001 (HS)
(x 10^4)	After	50	4.2566	3.6510	4.1134	
Colony	Before	50	1.3448	0.6695		0.0003 (HS)
Score	After	50	1.1875	0.7124	0.1573	

HS: Highly Statistically Significant



Graph 1: Distribution and comparison of the salivary *S.mutans* colony count (CFU/ml) in all the subjects before and after the administration of BTEMR.



Graph 2: Distribution and comparison of the salivary *S.mutans* colony score in all the subjects before and after the administration of BTEMR.

units /ml) and human blood (2% v/v) were added to this solution. It was then well mixed and poured equally into sterile petri dishes.

0.1ml of diluted 10^{-2}saliva sample was cultured on agar by spread plate technique. The plates were then incubated at 37°C for 48 hours in plastic bags inflated with expired air to enhance the carbon dioxide.

The number of colonies grown on the agar surface were counted with a magnifying glass in front of an illuminated source. Number of Colony Forming Units (CFUs) per ml of saliva was calculated as follows.
CFU in 0.001 ml = 'x' (Number of colonies)
Therefore, CFU/ml = 'x' × 1000
Thus, colony forming units (CFU)/ml of saliva was calculated and scored for each saliva sample.
Scoring of salivary *S. mutans* count:
0 < 10,000 CFU/ml
1 < 100,000 CFU/ml
2 = 100,000-1,000,000 CFU/ml
3 > 1,000,000 CFU/ml
CFU Colony Forming Unit.

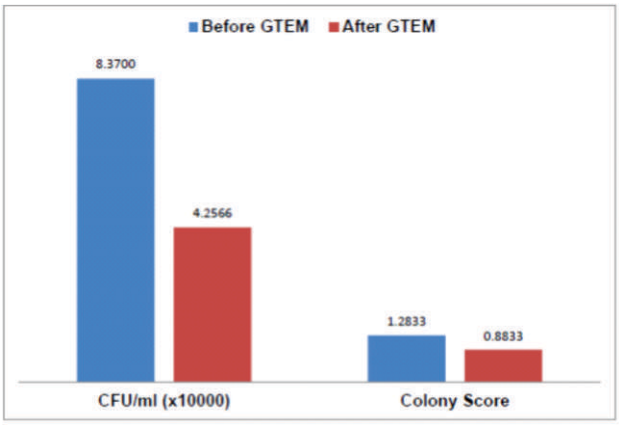
STATISTICALANALYSIS:

Paired *t* test was applied to analyse the difference in the CFU/ml. *p*-value of <0.05 was considered significant for the differences in the mean of CFU/ml and *S mutans* count scores before and after rinsing with BTEMR, CM or distilled water.

RESULTS:

The present study included a study group of 125 individuals in the age range of 21-40 years, divided into three groups and were administered BTEMR (n = 50), CM (n = 50), and distilled water (n = 25) Salivary samples were collected from each individual twice; the first sample which was collected prior to the administration of BTEMR, CM or distilled water, and the second sample was collected 30 min after the administration of BTEMR, CM or distilled water.

We compared *S.mutans* CFU/ml with the colony score before and after administration of BTEMR and found there was drastic reduction in colony score from 1.3448 to 1.1875. These results were highly significant [Table 1] [Graph 1, Graph 2 and Graph 3]. We found in our study that the results were almost similar when we compared the distribution and



Graph 3: Comparison of the mean salivary *S.mutans* load before and after the administration of BTEMR.

Table 2: Distribution and comparison of the salivary *S.mutans* colony count (CFU/ml) and the colony score in all the subjects before and after the administration of CM.

		N	Mean	Standard Deviation	Difference of means	*p*-value
CFU/ml (x 10^4)	Before	50	7.1700	9.0713	3.2134	<0.0001 (HS)
	After	50	3.9566	4.7630		
Colony Score	Before	50	1.1432	0.6787	0.2891	0.0002 (HS)
	After	50	0.8541	0.5612		

HS: Highly Statistically Significant

Table 3 : Distribution and comparison of the salivary *S.mutans* colony count (CFU/ml) and the colony score in all the subjects before and after the administration of distilled water.

		N	Mean	Standard Deviation	Difference of means	*p*-value
CFU/ml (x 10^4)	Before	25	8.2134	5.1533	0.6368	0.19 (NS)
	After	25	7.5766	6.8756		
Colony Score	Before	25	1.6332	0.9867	0.3978	0.21 (NS)
	After	25	1.2354	0.7586		

NS: Non Significant

colony score before and after the administration of CM and BTEMR. These results were also highly significant [Table 2]. However, when we used distilled water as the expectorant the results were not significant [Table 3].

DISCUSSION:

Among the various health issues being faced in day-to-day life, dental caries is one of the most common chronic diseases of modern times. Dental caries is associated with the frequency of fermentable carbohydrate intake. Also, certain dietary substances are more cariogenic than others. Simple sugars (e.g., Sucrose) are more cariogenic than complex sugars (e.g., Starch).[7]Caries is initiated by *S.mutans*, whereas Lactobacilli help further progression of the lesion inside the dentin.[8]Sakeenabi and Hiremath, Ravindran et al. and Pannu et al, concluded that *S.mutans* are the major pathogens responsible for dental caries.[9-12]

The microflora in dental caries is highly complex and varies between individual lesions. Mutans group Streptococci, such as *S.mutans* and Streptococcus sobrinus, and Lactobacilli are important in the initiation and progression of caries. These microorganisms are acidogenic (produce acid) by fermenting dietary carbohydrates, which result in the demineralization of enamel and dentin. They are also aciduric (acid tolerant), which gives them a competitive survival advantage.[8]

S.mutans and lactobacilli are regarded as the two chief bacterial species, responsible for decay in teeth. The relationship between oral cariogenic *S.mutans* and Lactobacilli species has been theorized by Sims. Studies of these organisms and their growth habits suggested that in most situations Streptococci are initial colonizers over teeth. The acid condition created by *S.mutans* favors the presence of lactobacilli. In presence of sucrose, *S. mutans* form extracellular dextrans and levans which can be utilized later to produce lactic acid.[8]Further, it is stated that *S.mutans* have a short generation time and multiply faster than lactobacilli. They are acidogenic, which results in accumulation of acid. As the pH falls, the generation time of *S.mutans* lengthens and becomes longer than lactobacilli. But when this occurs, Lactobacilli multiply more rapidly than Streptococci; the environment becomes more acidic and growth of *S.mutans* is inhibited. This show that caries is initiated by *S.mutans*, whereas lactobacilli help further progression of the lesion inside the dentin.[8]

Antimicrobial agents have been in the tradition that exert a direct bactericidal effect on caries producing bacteria. Many of these are the chlorhexidine mouthwashes,[13] gels, sodium hypochlorite solutions, etc. Their regular and long-term use cannot be advocated due to the potential side effects. Thus, there arises a need to introduce an agent that is relatively safe and equally efficient in targeting the microbial etiology. BTEMR was thus studied to know the antibacterial action that can be exerted by it on salivary load of *S.mutans*.

Black tea is a fermented tea harvested from the sp. Camellia sinensis. Major difference between black tea and the other types of tea is that black tea contains highest amount of catechins which are the major components possessing the antibacterial properties.[5]

These catechins make up almost 30 to 40% of the composition of dried black tea leaves [14-17]and so brewing only 2 g of these leaves in 100 ml of water (i.e. 2% of BTEMR) was done in the present study. This yielded a concentration of around 6000 to 8000µg/ml of the catechin compounds. These levels asserted to be well above the minimum inhibitory concentration of 250 to 1,000 µg/ml of the phenolic compounds of the black tea that is required to exert an antibacterial action.[18-20]

Black tea components are the polyphenols which constitute the most interesting group amongst the components of black tea leaves. The main polyphenols in black tea are catechins (flavan-3-ols). The four main catechins are: epigallocatechin 3 gallate (EGCG) that constitutes about 59% of total catechins, epigallocatechin (EGC) about 19%, epicatechin 3 gallate (ECG) about 13.6% and epicatechin (EC) about 6.4%.

The constituents of black tea synergistically help in the inhibition of dental caries and can be summarized into following mechanisms:

1. Remineralisation of dental hard tissues
2. Inhibition of bacterial enzymes
3. Prevention of bacterial adherence
4. Direct bactericidal action.

Lemos J (2005) suggested that the suppression of F1Fo- ATPase and agmatine deiminase system(AgDS) by EGCg may lead not only to energy starvation but also to disruption of constant pH across the cell membrane, which in turn may trigger a series of physiological effects in the cell. As a result of suppression of AgDS and F1Fo-ATPase cytoplasmic acidification and impaired acid tolerance may inhibit the normal function of various acid intolerant enzymes. The optimum pH range of GTFs in *S.mutans* was reported to be 5.5 to 6.0. The malfunction of GTFs at the lower pH value may lead to reduced production of EPS and intracellular polysaccharides (IPS). The latter of which could have been metabolized when exogenous fermentable substrate was depleted in the oral cavity. Therefore, the malfunction of GTFs may disrupt both bacterial adherence to the tooth surface and biofilm integrity and may augment the starvation stress of *S.mutans* cells due to the reduced preservation of IPS.[21] Cytoplasmic acidity, in concert with the inhibition of enolase by EGCg, may also inhibit the normal process of glycolysis, as described above. This in turn will diminish the ATP pool and further suppress the activity of the proton translocation (F1Fo-ATPase), aggravating cytoplasmic acidification. The inhibition of LDH at both transcriptional and enzymatic levels may also increase the levels of NADH and decrease the redox potential of the cell, leading to the NAD+ /NADH imbalance and/or accumulation of glycolytic intermediates in the cell, which is toxic for *S.mutans*. The net result would be cytoplasmic acidification and disrupted glycolytic processes with diminished ATP pools, thereby triggering a series of cascaded biological effects at molecular levels, leading to compromised competence to environmental stress and impaired cellular functions, even cell death. Thus, Xu X et al summarized that EGCg represents a natural and alternative anticariogenic agent because (i) EGCg inhibits growth of both *S.mutans* planktonic and biofilm cultures, and (ii) EGCg inhibits various cariogenic virulence factors of *S.mutans* at the transcriptional and enzymatic levels, leading to reduced acidogenicity and compromised stress tolerance (especially acid tolerance.

On comparison of the salivary load of *S.mutans* from the culture plates of samples obtained before and after the administration of BTEMR, it was observed that there was a significant fall in the salivary *S.mutans* load after rinsing with BTEMR.

The present study thus reflects the antibacterial effect of BTEMR particularly on salivary *S.mutans* and that regular use of BTEMR can keep a check on the major cariogenic microorganisms like *S.mutans* by exerting its bactericidal action.

The black tea consumption orally in the form of a drink, although being safe for most of the people in moderate quantities, few adverse health effects like gastrointestinal upset and hepatotoxicity are possible.[22]

CONCLUSION:

This study concluded that the effect of 2% BTEMR on salivary *S.mutans* load leads to significant reduction of the same irrespective of age or gender. This in turn implies that a regular use of BTEMR by the general population can prove to be an effective natural anticariogenic agent with no known implicated side effects.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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