



Original Research Article

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Effect of 0.5% Green Tea Leaves (*Camellia sinensis*) Mouth Rinse, 0.12% Chlorhexidine Mouth Rinse and Drinking Water on Pathogens of Dental Caries in School Children in Navi Mumbai

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ABSTRACT

Keywords

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Green tea (*Camellia sinensis*) is mouth rinse was prepared and its efficacy to use as a mouth wash is tested. Antibacterial and antifungal activity was compared with Chlorhexidine which is a Gold standard and plain drinking water mouth rinse was studied. Non stimulated saliva samples were tested using MSB agar for *S. mutans* and Rugosa agar for *Candida albicans*. Colony counts were done per ml of saliva and compared. There is no change in colony count in pre mouthwash and drinking water samples (Pre and Post). Green tea mouth wash rinse showed the effect by reducing the colony count and Chlorhexidine mouth rinse in comparison with Green tea or drinking water was found to be more effective. As chlorhexidine cannot be used regularly as it's a chemical, Green tea mouth wash has shown good antibacterial and antifungal activity and proved to be quite efficacious can be alternative to Chlorhexidine.

Introduction

The whole world is using plant based products as medicine since time immemorial. Humans have known many plants and their medicinal properties. Newer inventions and discoveries in medical and dental sciences made the way for dental cream and modified tooth brush including a battery operated tooth brush. The time has arrived for robotic brushes which cleans the teeth when instructions are given. There is a positive to this, if a patient is bed ridden but if he can operate the robot or with the others help, it will be useful to him/her. Chewing of neem and miswak twigs have

been in use without knowing the scientific value of it (Sorna Kumari *et al.*, 2011). In India, even now miswak tree and neem tree twigs are used as tooth cleaning tools even though toothbrushes have reached every corner of India through advanced dental sciences, awareness campaigns, audio and audio visual advertisements by manufacturers.

One more reason people use plant based products could be easy availability, decreased cost and the proven medicinal property of these plants. Use of these twigs gives double effect of tooth brushing as well as mouthwash as it leaves the residue after chewing of the

twigs. All these twigs have been proven to be having antimicrobial properties which helps in healing of gum diseases too.

In the present study, Green tea (*Camellia sinensis*) leaves extract was used in 0.5 % concentration in comparison with Chlorhexidine 0.12% which is a gold standard along with drinking water used as a control. Effect of all of these mouth rinses, pre and post unstimulated saliva cultures were done to cultivate *S. mutans* and *Lactobacilli* which are common cariogenic bacteria (Westergren and Krasse, 1978). *Streptococcus mutans* is considered as the major initiator pathogen for dental caries and classified into 3 serotypes C, E and F according to the different chemical composition of the serotype specific polysaccharides (World health Organization, 1997). A strong correlation has been established between dental caries and the salivary *Lactobacilli* count (Straetemans *et al.*, 1851-55).

Dental caries (Dye *et al.*, 2012) is one of the most common problems faced by children of school going age as they tend to eat more chocolates, sweets and do not brush their teeth after eating them. Maintaining good oral hygiene is one of the challenges faced by them. Bacteria tend to colonize or produce biofilm in the oral cavity. Normal flora of the oral cavity carries numerous bacteria and they are the etiologic agents for dental caries. Dental caries is the most common chronic infectious disease of childhood caused by the interaction of bacteria, mainly *Streptococcus mutans* and sugary foods on tooth enamel (Hakan Colak *et al.*, 2013). Regular and right tooth brushing techniques and use of mouth rinse is known to reduce the tooth decay as they have antibacterial activity. An effort has been made to grow the bacteria from salivary samples before the treatment and post test samples by growing the bacteria such as *Streptococcus mutans* and *Lactobacillus* Spp

and counting their colony forming units (CFUs).

Green Tea, non-fermented beverage is consumed around the world and has beneficial effects. Green tea is also used as a mouth gargle or mouthwash to treat dental decay, halitosis, laryngitis, mouth sores, plaque formation, sore throat, thrush and tonsillitis (Megalaa *et al.*, 2015). Chlorhexidine has been a 'gold standard' mouthwash liquid since its invention. Chlorhexidine gluconate mouthwash has earned the eponym of gold standard to treat and/or prevent periodontal disease. However, it has been reported to have local side effects on long term use (Simram Parwani *et al.*, 2003). Green tea has catechins which act as an antibacterial agent there by reducing the number of bacteria in the oral cavity which are responsible for dental caries. Effective use of green tea extract has been studied successfully in comparison with Chlorhexidine. Drinking water rinse was chosen as a neutral control so that we could get to know the changes in the number of colonies if any.

Materials and Methods

Study was started once the plan of the study was reviewed and approved by institutional ethical committee (IEC) of Bharati Vidyapeeth (Deemed to be University) Dental College & Hospital, Navi Mumbai. All children (participants) and parents were informed verbally in Marathi, Hindi and English and written informed consent was taken prior to the study. All the Microbiological studies were conducted at FEEV Research Lab, NaviMumbai.

135 school children of age group 7 to 10 years were selected by random selection after thorough dental check up by interns and Postgraduate students and Pedodontists. DMFT (decayed/missing/filled teeth ≥ 4)

status. Children were not on any antibiotics for 2 months prior to the start of the study. In a study it is concluded that, black tea and TM beverages exhibited strong antimicrobial effect against *S. mutans* and lactobacillus bacteria. Also, the moderate consumption of these beverages (2 cups/day) exhibited low values of bacterial counts. In addition it showed significantly lower dmf + DMF scores in beverage drinker children (Abd Allah *et al.*, 2012). Children were confirmed to be brushing their teeth at least once daily with non-fluoridated toothpaste. Subjects with any other dental problems, dental treatments like orthodontic treatment and / or oral prosthodontic devices were excluded. Instructions like brushing twice daily, i.e., once before breakfast with a gap of at least half an hour to act and maintain the activity and once before bedtime at night, using the mouth rinse. Mouth rinsing was done by the students for 15 consecutive days under the supervision by the dentist on working days and by parents on holidays/weekends.

Green tea leaves are handpicked from tea Gardens of Munnar, Kerala, and dried as per the instructions given by the Green Tea manufacturers/estate workers. These tea leaves are subjected to aqueous extraction by using Soxhlet method by keeping it for 24 to 48 hours. Freshly prepared extracts were given to the Dentist after cooling and by transferring it into leak-proof bottles to be given to the participants of the study. The constant touch with the participants, queries if any were answered which made the study easy.

Collection of the specimen

Approximately 3-5ml non-stimulated saliva samples were collected from each enrolled student under the supervision of dental interns/resident. Each student had to give two saliva samples, one is pre mouth wash sample and one more was after the use of mouth wash

after 15 days. The students were asked to keep the mouth wash for two minutes in the mouth for proper activity and this was repeated once daily for 15 days and then the Post-test saliva samples were collected.

Transportation

The saliva samples were collected in labelled sterile leak-proof containers and were transported to Microbiology laboratory and processed without delay (processed within 30 minutes of sample collection). The samples were processed at Five Elements Environmental Ventures Laboratory (FEEV), in Navi Mumbai.

Microbiological processing of samples

All the samples were subjected to bacterial culture in triplicates by inoculating 0.05 ml (50µl) of saliva samples each on MSB agar (Mitis Salivarius Bacitracin agar) (Sorna Kumari *et al.*, 2011) for *S. mutans* and on Rugosa agar for Lactobacillus Spp. All the labelled petri plates after inoculation in a laminar flow were incubated at 37⁰C incubator after keeping them in a Gas Pack Jar. Plates were incubated for up to 48 hours and colony count was made manually and electronically too for confirmation. Colony morphology was observed under naked eye and under stereo microscope too for confirmation. For every saliva samples average count was taken when counting CFU/ml.

Colony morphology: On MSB agar, granular bluish coloured colonies, which resemble ground glass appearance, were considered as *S. mutans*. The colony morphology was confirmed by hand lens and 5X and 10 X on compound microscope when needed. The strains were also confirmed by - a. Gram staining which showed Gram positive cocci in chains, b. Catalase test: negative c. sugar fermentation tests proved them to be

Streptococcus mutans. Sugar fermentation tests such as mannitol and sorbitol tests were done to confirm the bacterial isolates.

Lactobacilli spp. were cream coloured on Rugosa agar and were identified by colony morphology and Gram staining alone as on the Rugosa agar which is a selective media no other bacteria grew (To avoid contamination, all aseptic precautions are taken and samples were inoculated in Laminar air flow/Biological safety cabinets Level II (BSC Level II).

As all the petri plates were labelled. Growth has been recorded whenever samples are cultured. Once the all the samples were cultured/streaked, they were incubated at 37⁰C in Gas Pack jars upto 48 hours. After noting colony morphology and colony count, necessary tables were prepared in MS Office, MS Excel and MS Word and sent for statistical analyses.

Three groups were made of 45 subjects each. Group A used only 0.12% Chlorhexidine mouth rinse and Group B used 0.05% Green tree extract mouth rinse and Group C used drinking water as mouth rinse. None of the subjects missed the mouth washing during the active study period.

Statistical analysis

Statistical analysis was done by SPPSS software for degree of variance and P value which is found to be < 0.05.

Results and Discussion

Out of 135 patients unstimulated saliva samples tested, 135 samples were Pre mouth wash and 45 were post mouth wash of each 3 groups (A, B and C).

All 135 subjects each was supervised when different mouth rinses were used as a mouth wash. Each participant was aware of the good practices in dental hygiene like mouth rinses, tooth brushing etc. (Table 1).

Awareness drives were conducted before the recruitment of the participants

Table 2 shows that Pre mouth wash colony count was 10⁵CFU/ml of unstimulated saliva of all participants on an average. Post Chlorhexidine mouthwash/rinse showed significantly lower i.e., 10³ CFU/ ml and Green tea rinse too showed 10⁴ CFU/ml.

In drinking water post rinse samples, there was no change in the colony count (10⁵CFU/ml).

It's important to note that there was less number of colonies grown in post green tea rinse, and this trial is done for 15 days only which shows antibacterial and antifungal activity by green tea extract.

The results show there is a significant low number of CFUs isolated post rinse indicates there is a definite antibacterial and antifungal activity which in turn helps patients to keep their teeth healthy.

Table.1 Age wise and total numbers

Mouth rinse	Age (Mean)	N=135
Chlorhexidine (0.12%)	9.35	45
Green Tea (0.5)	9.41	45
Drinking water (control group)	9.38	45
Total	9.38	135

Table.2 Comparison of colony counts: n = 135

Bacteria isolated	No. of Colony forming units/ml			
	Pre mouth wash	Chlorhexidine	Green Tea	Drinking water rinse
<i>Streptococcus mutans</i>	1X10 ⁵	1X10 ³	1X10 ⁴	11X0 ⁵
<i>Lactobacilli Spp.</i>	1X10 ⁵	1X10 ³	1X10 ⁴	1X10 ⁵

Drinking water rinse has not changed the colony counts of the subjects tested. This shows that there is no effect of drinking water on the bacterial strains and counts even though solid particles might have been displaced in the mouth during the process. Chlorhexidine mouth wash is a gold standard among many a mouth washes available. Reduced colony count to significant levels of 10³ CFU/ml of saliva samples by use of Chlorhexidine. When compared to Chlorhexidine 10³ /ml, green tea (*Camellia sinensis*) mouthwash also showed significantly low numbers of colony count, 10⁴ /ml. This shows that effect of green tea mouth wash which is showing antibacterial and antifungal activity.

In this double-blind study, subjects and Microbiologist were not informed about the content of the mouthwash, and coded saliva samples are given for processing in Microbiology to eliminate any type of bias. All the saliva samples were processed immediately (within half an hour), so that the actual number of colonies would grow to achieve accuracy of actual colony count in the mouth. Otherwise there are possibilities of bacteria getting killed due to effects of saliva/ bacterial antagonism/ depletion in nutrition or overgrowth of other bacteria and yeasts like *Candida spp.*

Streptococcus mutans and *Lactobacilli spp* are isolated and identified which are potential pathogens and are known to cause dental caries. These cariogenic strains can be kept in check by using mouth rinses using green tea if 'Gold Standard' Chlorhexidine (0.12%) to be

avoided due to cost and chemical nature which may be harmful if used for long periods.

The results show effect of Green tea extract, which has shown significant reduction of number of bacterial colonies hence it can be used instead using only Chlorhexidine on a regular basis.

Green tea cost effective too and has antioxidant effects too.

The drinking water however does not show any changes in the colony count.

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