ANTICARIOGENIC POTENTIAL OF CAMELLIA SINENSIS ON STREPTOCOCCUS MUTANS- AN IN VITRO STUDY

Pubali Sarkar[1], Sandeep Kumar[2], Suranjita Das[3], Bhuvnesh Airen[4]

[1]Assistant Professor, Department of Dentistry, Jagannath Gupta Institute of Medical Sciences and Research, Kolkata, West Bengal, India.
[2]Lecturer, Department of Public Health Dentistry and Preventive Dentistry, Dental Institute, RIMS, Ranchi, Jharkhand, India.
[3]Senior Lecturer, Department of Conservative Dentistry and Endodontics, Mithila Minority Dental College and Hospital, Darbhanga, Bihar, India.
[4]Senior Lecturer, Department of Public Health Dentistry, Index Institute of Dental Sciences, Indore, Madhya Pradesh, India.

ABSTRACT

BACKGROUND

Dental caries is one of the most common chronic diseases of modern times. Chemical substances used for prevention of dental caries are known to have many side effects. Thus, natural products should be explored for their anti-caries action. We wanted to investigate the in-vitro antibacterial effect of tea leaves extract on Streptococcus mutans.

METHODS

This In Vitro experiment was conducted in the laboratory settings of Sri Aurobindo College of Pharmacy, Indore. Two types of tea leaves, black and oolong of a commercially available brand were used for the study. Aqueous extracts of both the tea leaves was prepared by boiling in distilled water in a standardized manner. Two concentrations 50% and 100%, were prepared and tested for antistreptococcal activity. Institute of Microbial Technology (IMTECH), Chandigarh supplied the bacterial strain Streptococcus mutans (MTCC 497) in lyophilized form. S.mutans was made viable by transferring into brain heart infusion (BHI) broth followed by incubation. Previously smeared S.mutans agar plates were then inoculated by the aqueous extracts of both the teas. Antimicrobial efficacy was checked using agar well diffusion method. The plates were incubated, and the zone of inhibition was determined. A similar procedure was carried out with Chlorhexidine 0.2%, which served as positive control. Statistical analysis was done using descriptive statistics and analytical tests like ANOVA to test the significance among variables.

RESULTS

Oolong as well as black tea aqueous extracts (100%), after an incubation of 48 hours hindered the growth of Streptococcus mutans. The efficacy of 100% aqueous extract was greater than 50% aqueous extract of both the types of tea. The mean zone of inhibition of oolong tea leaves aqueous extract (100%) was 19.18 ± 0.90 which was significantly higher than black tea 13.35±0.55 but it was less than chlorhexidine (0.2%) 24.80 ± 1.78 (p value<0.001).

CONCLUSIONS

A greater inhibitory effect was seen in Oolong tea extracts (Aqueous extract) compared to black tea (aqueous extract) on the growth of S.mutans.

HOW TO CITE THIS ARTICLE: Sarkar P, Kumar S, Das S, et al. Anticariogenic potential of camellia sinensis on streptococcus mutans-an in vitro study. J. Evolution Med. Dent. Sci. 2019;8(16):1268-1272, DOI: 10.14260/jemds/2019/283

BACKGROUND

Dental caries which is caused by Streptococcus mutans is a global oral health problem.[1] One of the main predisposing factors for the onset of dental caries is the presence of bacterial species that can lower the plaque pH to or below critical pH.[2] The correct approach would be, the prevention of caries formation.[3] The use of an antimicrobial agent apart from adequate practice of oral hygiene, can prevent the growth of microorganisms in the oral cavity. There has been an increased interest in the properties of some organic substances like chocolate, coffee, and tea, due to anticariogenic activity.[4]

'Financial or Other Competing Interest': None.
Submission 30-01-2019, Peer Review 11-04-2019,
Acceptance 17-04-2019, Published 22-04-2019.
Corresponding Author:
Dr. Pubali Sarkar,
#117 A,
Santosh Roy Road,
Kolkata-700008,
West Bengal, India.
E-mail: sarkar.pubali8@gmail.com
DOI: 10.14260/jemds/2019/283

Amongst those tea which originates in China has not only contributed the world's taste but has many pleiotropic beneficial properties including control of various oral pathogens especially S mutans.

All tea comes from the Camellia sinensis plant, what makes each tea different is the way it has been processed. Tea can be placed into 6 groups based on the amount of processing that goes into final product.

Types of Tea[5]

Based on the amount of processing that goes into final product, tea can be categorised as follows-

1. **Black Tea:** This tea goes through the most processing. Once the leaves are picked, they are left out in the sun to become slightly wilted. The leaves are then rolled to break open their tissue. The inner chemicals react with the air and begin to ferment. During the fermentation the leaves darken and change from green to red and finally to black. After the fermentation is complete, the leaves are then dried and packaged.

2. **Green Tea:** Green Tea is from the same plant as Camellia sinensis as all other teas. After the tea leaves are plucked and sorted, they are either steamed or pan fried. Green tea does not go through oxidation or fermentation process. The leaves are often rolled into different shapes before drying. Once the leaves are shaped, they are dried and packaged. Green tea also has HGCG; the most powerful antioxidant known.

3. **Oolong Tea:** Oolong Tea, like black tea goes through a withering stage (wilting).The difference is the oolong tea goes through a shorter stage and the leaves are fired directly after that to prevent continued oxidation (fermentation).The leaves can range from being almost black to dark green depending on when oxidation is stopped. The longer the leaves are oxidized the closer they will become to black tea. Formosa oolong is an amber oolong with a rich amber colour.

4. **White Tea:** It is the least processed tea. The leaves are picked early in the year while the tiny white hairs are still visible on the leaves and the bud is still closed. Only the top leaf and the bud are picked from the plant. The leaves are then allowed to dry in the sun. They are not steamed, or pan fired like green tea. If mechanical drying is required for a white tea, they are baked.[5]

Tea which is the second most commonly consumed beverage in the world is having per capita mean consumption in the world of approximately around 120 ml/day.[6] In India, the per capita consumption of tea annually is 706 g. There is an extensive literature suggesting medical health benefits associated with drinking tea (i.e., C sinensis).[7]

In India, the most favoured is Black tea while Green tea has been well researched. Oolong tea is specially consumed in china and Taiwan and reported to have medicinal effect including oral health and hygiene, but this tea is not consumed in India. The aim of the study was to investigate, in-vitro antibacterial effect of tea leaves extract on Streptococcus mutans with that of 0.2% chlorhexidine mouthwash.

Aims and Objectives

1. To assess the mean zone of inhibition of alcoholic & aqueous extract of oolong and black tea leaves on streptococcus mutans (in vitro)
2. To compare the mean zone of inhibition of Oolong and Black tea in 1:1, 1:2, 1:4, 1:8 dilution in (both aqueous and alcoholic) along with Chlorhexidine in same dilutions.

METHODS

The study was conducted in two parts. In the first part S mutans was made viable. The freeze –dried S mutans (MTCC 497) obtained from IMTECH (Institute of Microbial Technology, Chandigarh) was first transferred to brain heart infusion (BHI) broth and incubated for 24 hours to make S Mutans viable[8]

Then in the second part extract of both the leaves were prepared. Two different types of tea leaves (black and oolong tea of the same brand) were used in this study**.** This in vitro experiment was conducted in the laboratory settings of Sri Aurobindo College of Pharmacy; Indore.Ethical clearance was obtained from institutional review board. This study was approved as short study towards the partial fulfilment of requirements for the degree of (Master of Dental Surgery) by the Department of Public Health Dentistry, Sri Aurobindo College of Dentistry.

Preparation of Tea Extracts

Aqueous Tea Extracts[8]- Ten grams of each variety of tea was added to 100 ml of boiling distilled water and it was further boiled for 30 minutes. The extract obtained was reduced to 10 ml to obtain 100% (w/v) concentration. The extracts were cooled to room temperature prior to dilution.

Antibacterial Testing

The Bacterial sensitivity test was carried out in laminar air flow equipment using the disc diffusion technique. A suspension of Brain heart agar (BHI) infusion broth containing cultivated S mutans was placed on the centre of the Mueller Hinton Agar (MHA) plates. It was evenly spread on the plate using a sterile cotton swab. The antibacterial effect of aqueous and organic tea extracts was compared to that of 0.2% chlorhexidine. Fifty microlitres of each organic tea extract and 0.2% chlorhexidine was inoculated into the wells using micropipette. A total of 50 plates were prepared for two types of tea. After 24 hours of incubation, the petri dishes were observed for zones of inhibition i.e. areas without growth of S mutans.[9]

Statistical Analysis

Statistical analysis was done using SPSS Software (version 20.0).Data was analysed using ANOVA test, to know the difference between the groups. Post-hoc analysis was employed to specifically find that between which groups did significant difference existed. P value ≤ 0.05 was considered as significant.

Photograph Shows the Zone of Inhibition of The Growth of S Mutans in Different Dilutions of Black Tea, Oolong Tea (Alcoholic and Aqueous) and Chlorhexidine

Experimental Samples	Mean SD dil 1:1	Mean SD dil 1:2	Mean SD dil 1:4	Mean SD dil 1:8	p Value
Black tea (aq)	13.35 ± 0.55	12.35 ± 0.80	11.12 ± 0.34	0.00 ± 0.00	<0.001**
Black tea (alc)	12.17 ± 1.09	11.00 ± 0.66	0.00 ± 0.00	0.00 ± 0.00	<0.001**
Oolong tea (aq)	19.18 ± 0.90	11.68 ± 1.05	0.00 ± 0.00	0.00 ± 0.00	<0.001**
Oolong Tea (alc)	12.34 ± 0.85	11.27 ± 0.77	0.00 ± 0.00	0.00 ± 0.00	<0.001**
Chlorhexidine	24.80 ± 1.78	23.37 ± 1.42	22.52 ± 1.33	22.17 ± 1.10	<0.001**
p Value	<0.001**	<0.001**	<0.001**	<0.001**	

Table 1- Showing the Zone of Inhibition of Growth of S mutans With Different Dilutions Of Black Tea, Oolong Tea and Chlorhexidine

**p value<0.05 significant

RESULTS

The present study was carried out to find out the efficacy of the aqueous extract of oolong tea and black tea on Streptococcus mutans.

Table 1 shows the experimental samples of black tea aqueous, black tea alcoholic, oolong tea aqueous, oolong tea alcoholic extract. ANOVA test was applied which showed significant differences in intra and inter group comparison in all the dilutions of the 5 groups. There was significant difference in the zone of inhibition of chlorhexidine, black tea (Aqueous), black tea (Alcoholic), oolong tea (Alcoholic), oolong tea (Aqueous) in the dilution of 1:2, 1:4 & 1:8 respectively.

Post HOC Turkey Comparisons (Intergroup Comparisons)

	1:1 Dilution				
	Black (aq)	**Black (alc)**	**Oolong (aq)**	**Oolong (alc)**	**Chx**
Black (aq)	-	.144	<0.0001**	.272	<0.0001**
Black(alc)	.144	-	<0.0001**	.997	<0.0001**
Olg (aq)	<0.0001**	<0.0001**		<0.0001**	<0.0001**
Olg (alc)	.272	.997	<0.0001**	-	<0.0001**
Chx	<0.0001**	0.000	<0.0001**	<0.0001**	-

Table 2- Showing Post Hoc Tukey Comparison of Zone of Inhibition of Growth of S mutans With Different Dilutions of Black Tea, Oolong Tea and Chlorhexidine

**p value<0.05 significant

Table 2 Shows intergroup comparisons between black (Aqueous), black (Alcoholic), oolong (aq), oolong (alc) in 1:1 dilution. A significant difference was observed when black (aq) was compared with oolong (aq) (p value<0.0001) and with chlorhexidine (p value <0.0001).While on comparing oolong (aq) with black (aq), black (alc), oolong (alc) & chlorhexidine respectively a significant difference (p value <0.0001) was obtained. Oolong (alc) showed significant difference with oolong (aq) & chlorhexidine (p value <0.0001). Chlorhexidine showed significant difference with black (aq), oolong (aq) & oolong (alc) (p value <0.0001).

	1:2 Dilution				
	Black (aq)	**Black (alc)**	**Oolong (aq)**	**Oolong (alc)**	**Chx**
Black (aq)	-	.28	.553	.119	<0.0001**
Black(alc)	.028	-	.539	.972	<0.0001**
Olg (aq)	.553	.539	-	0.883	<0.0001**
Olg (alc)	.119	.972	.883	-	<0.0001**
Chx	<0.0001**	<0.0001**	<0.0001**	<0.0001**	-

Table 3. Post HOC Turkey Comparisons (Intergroup Comparisons)

**p value<0.05 significant

Table 3 Shows intergroup comparisons at 1:2 dilution of Black (aq), Black (alc), oolong (aq), oolong (alc).A significant difference was observed when Black (aq), Black (alc), oolong (aq), oolong (alc) was compared with chlorhexidine (p value <0.0001).

	1:4 Dilution				
	Black (aq)	**Black (alc)**	**Oolong (aq)**	**Oolong (alc)**	**Chx**
Black (aq)	-	<0.0001**	<0.0001**	<0.0001**	<0.0001**
Black (alc)	<0.0001**	-	1.000	1.000	<0.0001**
Olg (aq)	<0.0001**	1.000	-	1.000	<0.0001**
Olg (alc)	<0.0001**	1.000	1.000	-	<0.0001**
Chx	<0.0001**	<0.0001**	<0.0001**	<0.0001**	-

Table 4. Post HOC Turkey Comparisons (Intergroup Comparisons)

**p value<0.05 significant

Table 4 shows intergroup comparisons between Black (aq), Black (alc), Olg (aq), and Oolong (alc) at 1:4 dilution. Black (aq) showed significant difference with black (alc), oolong (aq), oolong (alc) and chlorhexidine (p value <0.0001).Black (alc), Olg (aq), Olg (alc) showed significant difference with Black (aq), Black (alc), Oolong (aq), Oolong (alc) & chlorhexidine. (p value <0.0001).

	1:8 Dilution				
	Black (aq)	**Black (alc)**	**Oolong (aq)**	**Oolong (alc)**	**Chx**
Black (aq)	-	<0.0001**	1.000	1.000	<0.0001**
Black (alc)	1.000	-	1.000	1.000	<0.0001**
Olg (aq)	1.000	1.000	-	1.000	<0.0001**
Olg (alc)	1.000	1.000	1.000	-	<0.0001**
Chx	<0.0001**	<0.0001**	<0.0001**	<0.0001**	-
Table 5. Post HOC Turkey Comparisons (Intergroup Comparisons)					
**p value<0.05 significant					

Table 5 Shows intergroup comparisons between black (aq), Black (alc), Oolong (aq), Oolong (alc), Chlorhexidine at 1:8 dilution. Black (aq) showed significant difference with black (alc) and chlorhexine (p value<0.0001).Black (alc) showed significant difference with chlorhexine (p value<0.0001).Oolong (aq) & oolong (alc) showed significant difference with chlorhexidine (p value <0.0001).Chlorhexidine showed significant difference with Black (aq), Black (alc), Oolong (aq), Oolong (alc) (p value<0.0001).

DISCUSSION

In this experimental study, antimicrobial efficacy of extracts of tea leaves (black & oolong) were tested against Streptococcus mutans. Tea has a chemical composition of polyphenols, amino acids, enzymes, pigments, carbohydrates, methylxanthines, minerals, and many volatile compounds.[10]

These compounds are responsible for producing teas with desirable appearance, aroma and taste. Thus, this study attempted to evaluate the efficacy of tea leaves, a rich source of antibacterial substances against caries causing Streptococcus mutans.

Various epidemiological studies have been conducted to check the efficacy of green tea on the growth of S mutans, however limited studies have been conducted to check the efficacy of black tea against S mutans.[11] Our study was different as the efficacy of both oolong and black tea against S mutans was carried out in the laboratory. In this study also, different dilutions like 1:1, 1:2, 1:4 and 1:8 were used. The study findings showed that aqueous extract of oolong tea leaves was significantly better than black tea at higher concentrations.

However, no significant difference was found at lower concentrations. This suggests that aqueous extract of oolong tea has good antibacterial properties against S mutans only at higher concentrations. No significant difference was observed between alcoholic extract of black and oolong tea at all the concentrations. Various studies have shown the efficacy of chlorhexidine against streptococcus mutans.[12]

However, on contrary, a study conducted by Subramaniam et al.[13] in 2012 reported that (aqueous and organic) Oolong tea extracts prevented the growth of S. mutans better than chlorhexidine. In our study, chlorhexidine was found to be better & effective than oolong and black tea leaves extract. Chlorhexidine, a cationic bisbiguanide with a very broad antimicrobial spectrum is the most widely used over the counter mouth rinse for cleaning procedures as well as used alone. Its effectiveness was also shown for control of gingivitis in long term studies. Therefore, Chlorhexidine was taken as a control.

Nagappan N et al.,[14] in his study compared the antimicrobial efficacy of a herbal & chlorhexidine mouth wash against Streptococcus mutans. This was measured by zone of inhibition and minimum inhibitory concentration. Dalinsali Z et al (2011).[15] Hasanali et al (2012).,[16] Fereshten et al (2012).,[17] Pooja Agarwal et al (2010),.[18] and Vijaya Hegde et al (2011),[19] compared the antimicrobial efficacy of different herbs with chlorhexidine mouthwash against S mutans and found satisfactory result with chlorhexidine than any other herbs.

In oral applications, chlorhexidine binds to the mouth tissue, oral mucosa and teeth. It is the released over time to kill bacteria and fungi. This helps to reduce the bacterial count and prevents dental plaque.[20]

The results of the present study strongly suggest that certain components of tea exert a preventive effect against S. mutans. The present study suggests that drinking oolong tea is more effective than black tea in the prevention of dental caries. Estimation of the biologically active compounds in tea extracts should be done in future. Further evaluation of tea extracts would probably corroborate these findings. One probable limitation of this study is it was performed on standard microorganisms so further studies on oolong and black tea effects on mouth derived Streptococcus mutans or in vivo experiment are necessary.

In this study concentration of 50% and 100% has been used which is practically not feasible but it can be considered a functional food for oral health by controlling through prevention the most prevalent infectious disease of mankind: caries.

CONCLUSIONS

Chlorhexidine was found to be more effective than both the alcoholic and aqueous extracts of black & oolong tea at all concentrations. Chlorhexidine was more effective at higher concentration than when diluted. It still remains the gold standard for antibacterial properties against S mutans compared to black and oolong tea leaves extracts. It is always difficult to translate diverse scientific findings into public health messages, yet it is a logical step; but evidence suggests an intake of at least three cups of black tea a day is ideal for CHD prevention; one to six cups per day for significant increases in plasma antioxidant capacity and less than eight cups of tea per day to avoid adverse effects on hydration and iron status (to manage intakes of caffeine and phenolic compounds from tea). For cancer, bone health and dental health, there was insufficient evidence to make any recommendations about intakes. Hence, further research is needed to check the efficacy of tea leaves' extracts against S.mutans.

REFERENCES

[1] Loesche WJ. Role of Streptococcus mutans in human dental decay. Microbiol Rev 1986;50(4):353-80.

[2] Ooshima T, Osaka Y, Sasaki H, et al. Caries inhibitory activity of cacao bean husk extract in vitro and animal experiments. Arch Oral Biol 2000;45(8):639-45.

[3] Nakahara K, Kawabata S, Ono H, et al. Inhibitory effect of oolong tea polyphenols on glycosyltransferases of mutans streptococci. Applied and Environmental Microbiology 1993;59(4):968-73.

[4] Rietveld A, Wiseman S. Antioxidant effects of tea: evidence from human clinical trials. J Nutr 2003;133(10):S3285-S92.

[5] Balentine DA. Manufacturing and chemistry of tea. In: Ho CT, Huang MT, Lee CY, eds. Phenolic compounds in food and their effects on health I: analysis, occurrence and chemistry. Vol. 8. Washington DC: American Chemical Society 1992: p. 102-17.

[6] Estimates of (internal) consumption and per capita consumption of tea in India. http://teaboard.gov.in/pdf/stat/Consumption.pdf. (Last accessed on 19.1.2015).

[7] McKay DL, Blumberg JB. The role of tea in human health: an update. J Am Coll Nutr 2002;21(1):1-13.

[8] Subramaniam K, Siswomihardjo W, Sunarintyas S. The effect of different concentrations of Neem (Azadiractha indica) leaves extract on the inhibition of Streptococcus mutans (In vitro). Maj Ked Gigi (Dent J) 2005;38(4):176-9.

[9] Sherwani SK, Bashir A, Haider SS, et al. Thrombolytic potential of aqueous and methanolic crude extracts of camellia sinensis (green tea): in vitro study. J Pharmacogn & Phytochem 2013;2(1):125-9.

[10] Karori SM, Wachira FN, Wanyoko JK, et al. Antioxidant capacity of different types of tea products. Afr J Biotechnol 2007;6(19):2287-96.

[11] Lee KW, Lee HJ, Lee CY. Antioxidant activity of black tea versus green tea. J Nutr 2002;132(4):785.

[12] Emilson CG. Potential efficacy of chlorhexidine against mutans streptococci and dental caries. J Dent Res 1994;73(3):682-91.

[13] Subramaniam P, Eswara U, Reddy MKR. Effect of different types of tea on Streptococcus mutans: an in vitro study. Indian J Dent Res 2012;23(1):43-8.

[14] Nagappan N, John J. Antimicrobial efficacy of herbal and chlorhexidine mouth rinse - a systematic review. J Med Dent Sci 2012;2(4):5-10.

[15] Dalirsani Z, Aghazadeh M, Adibpour M, et al. In vitro comparison of the antimicrobial activity of ten herbal extracts against streptococcus mutans with chlorhexidine. Journal of Applied Sciences 2011;11(5):878-82.

[16] Shafiee H, Mortazavi H, Baharvand M, et al. Iranian traditional medicine: comparison of the antibacterial effect of ANNAS 0.2% and chlorhexidine 0.2%. African Journal of Microbiology Research 2012;6(15):3600-03.

[17] Sedighinia F, Safipour AA, Soleimanpour S, et al. Antibacterial activity of Glycyrrhiza glabra against oral pathogens: an in vitro study. Avicenna Journal of Phytomedicine 2012;2(3):118-24.

[18] Agarwal P, Nagesh L, Murlikrishnan. Evaluation of the antimicrobial activity of various concentrations of Tulsi (ocimum sanctum) extract against streptococcus mutans: an in vitro study. Indian Journal of Dental Research 2010;21(3):357-9.

[19] Hegde V, Vasavi. A study to assess the antibacterial effect of Triphala churna against streptococcus mutans-an in vitro study. Journal of Postgraduate Dentistry 2011;1(2):13-4.

[20] Bansal S, Kapoor D, Bharti V. Chlorhexidine mouth rinse: a review. J Indian Dent Assoc 2001;72:331-3.