AYU

Access this article online
Website: www.ayujournal.org
DOI: 10.4103/0974-8520.182743
Quick Response Code:

Clinical Research

Inhibition of salivary amylase by black tea in high-caries and low-caries index children: A comparative *in vivo* study

Vishal Arya, Lavina Taneja[1]

Departments of Pediatric and Preventive Dentistry and [1]Oral Medicine Diagnosis and Radiology, Faculty of Dental Sciences, SGT University, Budhera, Gurgaon, Haryana, India

Abstract

Introduction: Dental caries is a universal disease. Dietary modification is important in reducing the occurrence of dental caries. Tea which is so frequently consumed with cariogenic starch rich food is proposed to have anticariogenic potential. The various mechanism has been proposed for same and one being inhibition of salivary amylase activity. **Aim:** To determine the effect of 1.5% black tea decoction on salivary amylase activity in children with high caries and no caries. **Materials and Methods:** A total of 30 children in the age group of 12–15 years were selected for the study. They were further grouped based on their decayed missing filled surface (DMFS) score into high-caries group (DMFS above 10) and no-caries group (DMFS = 0). After 2 h of fasting, subjects consumed two salted crackers for 60 s following which they rinsed with water and then with black tea decoction (1.5%) the very next day. Retained food particles were recovered salivary amylase activity was noted as maltose to sucrose ratio via chromatography. **Results:** The average ratio of maltose to sucrose ratio percentage reduction in high-caries group was 43.63% and 41.17% in no caries group which was highly significant ($P < 0.005$) while the intergroup comparison was found statistically insignificant. **Conclusions:** Tea decoction has inhibitory effect on salivary amylase activity thus dental caries. The effect was statistically insignificant in children with high- and no-caries index.

Key words: Amylase, black tea, dental caries

Introduction

Dental caries is a widely spread dietobacterial disease involving demineralization of inorganic and destruction of the organic component of enamel and dentin. Researcher at the National Institute of Dental Research reported as a result of a series of experiments, which revolutionized the thinking about dental caries for decades to come.[1] The important components of this triad are susceptible host, cariogenic diet, and bacteria. This disease *per se* is resistant to antimicrobial therapy. Dietary components such as sucrose and starch are well implicated in the etiology while certain food components are considered to be caries protective. Teas are known for its anticariogenic action.[2-4] One of the proposed mechanism being inhibition of salivary amylase which inturn reduces the cariogenic potential of starch via reducing the release of fermentable carbohydrates.[5] There can be variation in the inhibition of this enzyme activity *in vivo* between high caries and no caries group. Apparently, this area holds future prospects as one of the preventive mechanisms from an epidemic of dental caries.

Materials and Methods

The study was carried out in the Department of Pediatric Dentistry at Nair Hospital Dental College, Mumbai. The study was in accordance with Helsinki's declaration. Participation in the study was voluntary, and ethical clearance was obtained for the study. The procedure was explained in details to the

Address for correspondence: Dr. Lavina Taneja,
A - 150, Second Floor, Lokvihar, Pitampura,
New Delhi - 110 034, India.
Email: aryalavina@gmail.com

How to cite this article: Arya V, Taneja L. Inhibition of salivary amylase by black tea in high-caries and low-caries index children: A comparative *in vivo* study. Ayu 2015;36:278-82.

respective parents or guardians, and informed consent was obtained from them. A total of 500 children in age group of 12–15 years were screened to evaluate their dental caries status as per the WHO criteria (1997).[6]

Inclusion criteria

The children in age group of 12–15 years and with good general health were included in the study.

Exclusion criteria

Medically compromised children or one with a history of hospitalization or intake of antibiotics or medication in the past 6 months or not willing to take part in study were excluded from the study.

Grouping

Subjects fulfilling inclusion and exclusion criteria were randomly categorized by lottery method into two groups based on decayed missing filled surface (DMFS) score, i.e., Group 1: children with high caries (DMFS score more than 10) and Group 2: children with no caries (DMFS score zero). The two groups were age and sex matched.

Posology

Tea decoction was prepared by suspending 3 g of black tea powder in 200 ml of distilled water at 100°C, stirring gently for 3 min, and filtering through Whatman's filter paper no. 1. A 1.5% solution of tea was thus prepared.

Estimation of tannins

Tannins were estimated in the following sequence.

Preparation of standard curves of gallic acid

The method of Ragazzi and Veronese[7] was used. Total phenolic content, collectively known as Tannins, was measured as gailic acid (GA) equivalents, using Folin and Ciocalteu's phenol reagent. A standard curve of gallic was thus prepared using standard dilutions of GA ranging from 5 μg/ml to 75 μg/ml. 1 ml each of the GA solutions (serial dilutions) were diluted to 50% of original concentration with menthol, 0.5 ml of Folin, and Ciocalteu's phenol reagent (2.0N), and 3.0 ml of Na_2CO_3 (200 mg/ml) were mixed in order. The mixture was vortexed and the reaction allowed to proceed for 15 min at room temperature. The reaction mixtures were diluted with 10 ml of deionized water. A white precipitate that formed from Folin and Ciocalteu's Phenol reagent was removed by centrifuging for 5 min at 1250 G. Absorbance of the supernatants were measured at 725 nm in the spectrophotometer.

Estimation of total phenolic content of tea decoction

Tea decoction (1.5%) was prepared in the manner stated before. It was centrifuged at 3000 G at room temperature to remove any particulate matter or dust particles. Serial dilution of tea extract was then made in three ranges: 1:100, 1:50, and 1:25 (two samples each), using methanol as control and GA as standard and serial dilutions of tea as test solutions. Total phenol content of tea decoctions was determined. The method used was that of Ragazzi and Veronese,[7] as described before.

Estimation of soluble phenolic content of tea decoction

The crude extract of tea (1.5%) was prepared in as similar fashion. It was treated with equal volume of 0.5% calf gelatin solution and centrifuged at 5000 G for 15 min at room temperature. This led to precipitation of condensable tannins while soluble tannins remained in supernatant. Three serial dilutions of the supernatant were made, 1:100, 1:50, and 1:25 using methanol as control, GA as standard, and serial dilutions of gelatin treated tea decoction as test solution, soluble tannin concentration of tea extract decoction was estimated spectrophotometrically and reading absorbance at 725 nm.

Collection of samples

Two hours of fasting period was a requirement, before the collection of food samples. Each subject was given 2 salted crackers (2.8 g each with maltose to sucrose ratio of 0.4) to chew approximately 60 s following which, they rinsed with 20 ml water for 20 s. Retained food was recovered from the buccal surface of left mandibular first or second premolar after 3 min using curette and placed in 1 ml distilled water in microcentrifuge tubes. The caps were closed, and tubes were placed in boiling water for 5 min to deactivate amylase. The same subjects were called the next day, and the same procedure was repeated using 20 ml tea decoction at room temperature (1.5% solution with tannin concentration of 1.59 mg/ml).

Storage of samples

The tubes, immediately after sample collection, were carried in a chilled container to the laboratory, where they were stored at −23°C for further chromatographic analysis.

Chromatographic analysis

For chromatographic analysis, samples were thawed and brought to room temperature. The tubes were then centrifuged at 1500 g for 5 min, and resultant supernatant fluid was diluted 5 folds with deionized water. Fifty microliters aliquots of diluted samples were used for chromatographic analysis of maltose and sucrose using Dionex high-performance anion exchange liquid chromatography system, with CarboPac PA1 column. NaOH was used as etrant. Gradient varied from 30–70 to 8–92 mv. Isocratic elution with 0.1 Smol/L NaOH and pulsed amperometric detection of separated sugars was carried out. The Maltose to sucrose ratios of both the groups was assessed from chromatograms. The results obtained were analyzed statistically.

Statistical analysis

Student *t*-test was used for comparison of results. The statistical significance was set at $P < 0.005$.

Results

In an entrapped particle of food, the amount of sucrose tends to remain constant as long as particles are retained on dentition but the amount of maltose increases due to the action of salivary amylase on starches. Maltose to sucrose ratio was thus used to evaluate the results. In our study, all 30 subjects successfully completed the study.

In Group 1, i.e., with high-caries group, average maltose to sucrose ratio obtained using water as rinsing solution was 3.25

and 1.82 with 1.5% black tea decoction (1.5%). An average reduction of 43.49% in maltose to sucrose ratio was found. Statistically, the reductions obtained were highly significant, i.e. $P < 0.005$ [Tables 1 and 2].

In Group 2, i.e. no caries group, it was found that the average maltose to sucrose ratio was 3.30 when water was used as rinsing solution and 1.83 when black tea decoction. An average reduction of 41.25% in maltose to sucrose ratio was found and it was highly significant ($P < 0.005$) [Tables 1 and 2].

However no statistically significant differences, in the reductions of maltose to sucrose ratios were found ($P > 0.005$) when intergroup comparisons were made [Table 2].

Discussion

Dental caries is a widely researched area in dentistry which has proved to be heterogeneous disease complex with multiple factors contributing to its initiation and expression. The etiology is multifactorial with the interplay of suitable substrate, susceptible host, and microorganism. Dietary carbohydrates, especially sucrose and sticky foods like chips, chocolates, and starch-rich diet are universally accepted as the etiological agents. Thus, dietary modification and substances having anticariogenic activity can play an important role in its prevention.

Tea is obtained from dry leaves of *Camellia sinensis* is known for such effect. Moreover, it is a most commonly used beverage in the world after water thus the use is widespread. A number of mechanisms have been invoked to explain this effect.[2,8] These include the influence of endogenous tannins on bacterial growth and viability,[9-11] the inhibition of glucosyl transferase,[12] high-fluoride content of tea, action on salivary amylase.

Salivary alpha-amylase is the most plentiful and physiologically active enzyme in the oral cavity. It has three distinct functions, i.e. its enzymatic action plays a role in carbohydrate digestion, amylase in solution binds with high affinity to selected group of streptococci and also plays a role in adhesion of alpha-amylase binding bacteria.[13-16]

Table 1: Comparison of Maltose/Sucrose ration in both the groups (*n*=15 in each group)

Group	Maltose/sucrose ratio after		Difference	*t* value	*P* value
	Water rinses	Tea rinses			
1	3.258±0.681	1.823±0.123	1.435±0.514	10.802	<0.001
2	3.306±1.645	1.832±0.740	1.474±1.022	5.584	<0.001

Data: Mean±SD

Table 2: Comparison of percentage reduction in maltose to sucrose ratio (*n*=15 in each group)

% Change in maltose/sucrose ratio		Difference	*t* value	*P* value
Group 1	Group 2			
43.491±11.474	41.251±10.501	2.241	0.558	0.581

Data: Mean±SD

All these activities depend on an intact enzyme configuration. Amylase bound to bacteria in plaque as well as free amylase in saliva may facilitate dietary starch hydrolysis to provide additional low-molecular weight carbohydrates, for metabolism by micro-organisms. The resulting lactic acid produced may add to the pool of acid in plaque to contribute to further tooth demineralization thus resulting in further progression of dental caries.[13]

Salivary amylase plays a catalytic role in a random splitting of α-1,4 glucosidic bonds of glucan. The end products of this reaction are maltose, some glucose and limit dextrins.[15,17-19] Thus, salivary amylase enzyme catalyzes the hydrolytic cleavage of food starch to maltose and other low molecular weight carbohydrates which act as a substrate for the development of dental caries.

Thus, we designed our study to know whether black tea decoction can have any effect on salivary amylase activity and if there is any variation in the effect in children with high and no caries. The 12–15 years age group was selected so that all the permanent teeth had been exposed to the oral environment for a sufficient period and to have a true picture of the permanent dentition.

The result of present study state that the salivary amylase activity is significantly inhibited by black tea decoction rinse both in high caries and no caries group. The study results are in consensus with the previous study[20] which reported the similar effect on salivary amylase activity via intraoral hydrolysis of starch shortly after consumption of black and green tea. 1% tea decoction was used to asses *in vitro* activity. Further *in vivo* experiment was done on few subjects and sample was collected rapidly, i.e., 0.5, 1 and 1.5 min in contrast to our study in which only 1.5% black tea decoction was used and the sample was retrieved after 3 min. Above study showed greater inhibitory effect which can be explained by delayed collection of the sample during which effect may have reduced. The suppression of salivary amylase activity from *Streptococcus mutans* by both black as well as green tea was also reported.[20] A study done on 10 healthy subjects using tea rinses for varying 5 and 10 times and with water as control shows rinsing with black tea infusion 10 times a day resulted in fall of pH ($P < 0.05$), lower plaque index and higher fluoride concentration in plaque and saliva.[21] Another research also reported the anti-enzymatic effect of black tea because of high-molecular weight of polyphenols and the aflavin.[22]

Salivary amylase enzyme which is present in saliva as well as produced by a microorganism present in the oral cavity. Although it has important role in the development of dental caries the correlation between the level of this enzyme and incidence of dental caries is inconclusive.[23] This can be a possible explanation for the insignificant difference in the inhibitory effect of black tea between high caries and no caries group in our study.

We further recommend studies with larger sample size and more lifelike situation, e.g. withdrawing food samples at regular specified time intervals during normal tea drinking situation in human subjects and evaluating for maltose and maltotriose content as it can open gateways to the effective and widespread caries preventive strategies worldwide. Moreover, limitation of

our study is that high-fluoride level of black tea is also proposed as a mechanism for caries prevention which was not assessed in present study.[4]

Conclusion

Alpha-amylase is the key enzyme responsible for fermentation of starches to low-molecular substrate for dental caries. Black tea decoction rinses showed the significant inhibitory effect on this enzyme irrespective of their prior caries status. Thus, the effect of tea on amylase may be considerably significant in reducing the cariogenicity of starch-containing foods that are so frequently consumed with tea.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Keyes PH. The infectious and transmissible nature of experimental dental caries. Findings and implications. Arch Oral Biol 1960;1:304-20.
2. Otake S, Makimura M, Kuroki T, Nishihara Y, Hirasawa M. Anticaries effects of polyphenolic compounds from Japanese green tea. Caries Res 1991;25:438-43.
3. Wu-Yuan CD, Chen CY, Wu RT. Gallotannins inhibit growth, water-insoluble glucan synthesis, and aggregation of mutans streptococci. J Dent Res 1988;67:51-5.
4. Goenka P, Sarawgi A, Karun V, Nigam AG, Dutta S, Marwah N. Camellia sinensis (Tea): Implications and role in preventing dental decay. Pharmacogn Rev 2013;7:152-6.
5. Leeds AR. Black tea-helpful or harmful? A review of the evidence. Eur J Clin Nutr 2007;61:3-18.
6. Oral health surveys — Basic methods. 4th Edition. Geneva: World Health Organization; 1997.
7. Ragazzi E, Veronese G. Quantitative analysis of phenolic compounds after thin-layer chromatographic separation. J Chromatogr 1973;77:369-75.
8. Elvin-Lewis M, Steelman R. The anticariogenic effects of tea drinking among Dallas school children. J Dent Res 1986;65:198.
9. Sakanaka S, Shiumua N, Masumi M, Kim M, Yamamoto T. Preventive effect of green tea polyphenols against dental caries in conventional rats. Biosci Biotechnol Biochem 1992;56:592-4.
10. Kumar PV, Shruthi BS. Tea: An oral elixir. Sch Acad J Pharm 2014;3:9-18.
11. Allah AA, Ibrahium MI, Al-Atrouny AM. Effect of black tea on some cariogenic bacteria. World Appl Sci J 2011;12:552-8.
12. Kakuichi N, Hattori M, Nishizawa M, Yamagishi T, Okuda T, Namba T. Studies on dental caries prevention by traditional medicine: Inhibitory effect of various tannins on glucan synthesis by glucosyltransferase from *Streptococcus mutans*. Chem Pharm Bull 1986;34(2):720-5.
13. Scannapieco FA, Torres G, Levine MJ. Salivary alpha-amylase: Role in dental plaque and caries formation. Crit Rev Oral Biol Med 1993;4:301-7.
14. Hu Y, Ruan M, Wang Q. A study of parotid salivary proteins from caries free and caries active people by high performance liquid chromatography. Chung Hua Kou Ching Hsueh Tsa Chih 1997;32:95-8.
15. Koyama I, Komine S, Yakushijin M, Hokari S, Komoda T. Glycosylated salivary alpha-amylases are capable of maltotriose hydrolysis and glucose formation. Comp Biochem Physiol B Biochem Mol Biol 2000;126:553-60.
16. Shore RC, Kirkham J, Brookes SJ, Wood SR, Robinson C. Distribution of exogenous proteins in caries lesions in relation to the pattern of demineralisation. Caries Res 2000;34:188-93.
17. Whelan WJ, Roberts PJ. Action of salivary alpha-amylase on amylopectin and glycogen. Nature 1952;170:748-9.
18. Jacobsen N, Melvaer KL, Hensten-Pettersen A. Some properties of salivary amylase: A survey of the literature and some observations. J Dent Res 1972;51:381-8.
19. Mörmann JE, Mühlemann HR. Oral starch degradation and its influence on acid production in human dental plaque. Caries Res 1981;15:166-75.
20. Zhang J, Kashket S. Inhibition of salivary amylase by black and green teas and their effects on the intraoral hydrolysis of starch. Caries Res 1998;32:233-8.
21. Lingstrom P, Wu CD, Wefel JS. *In vivo* effects of black tea infusion on dental plaque. J Dent Res 2000;76:593.
22. Hara Y, Honda M. The inhibition of alpha amylase by black tea polyphenols. Agric Biol Chem 1990;54:1939-45.
23. Birkhed D, Skude G. Relation of amylase to starch and Lycasin metabolism in human dental plaque *in vitro*. Scand J Dent Res 1978;86:248-58.

हिन्दी सारांश

उच्च और अल्प क्षय सूचकांक बच्चों में काली चाय से सलाईवरी एमाईलेज पर प्रभाव – एक तुलनात्मक अध्ययन

विशाल आर्य, लविना तनेजा

दंत क्षय एक सार्वभौमिक बीमारी हैं। खान–पान का संशोधन दंत क्षय की घटना कम करने में महत्त्वपूर्ण है। काली चाय अक्सर दंत क्षय जनक स्टार्च युक्त भोजन के साथ सेवन किया जाता है, जो दंत क्षय निषेध क्षमता के लिए प्रस्तावित किया गया है। सलाईवरी एमाईलेज गतिविधि रोधक तंत्र प्रस्तावित है। वर्तमान अध्ययन का उद्देश्य उच्च क्षय और अल्प क्षय गतिविधि बच्चों में सलाईवरी एमाईलेज गतिविधि पर १.५% काली चाय के काढ़े का प्रभाव निर्धारित करना है। १२–१५ साल वर्ष की आयु वर्ग के ३० बच्चों को अध्ययन के लिए चयन किया गया। वे आगे उच्च क्षय समूह (१० से ऊपर डीएमएफ) और कम क्षय समूह (शून्य डीएमएफ) में स्कोर के आधार पर वर्गीकृत किये गये। दो घंटे उपवास उपरान्त बच्चों को ६० सेकंड के लिए निश्चित खाद्य समान रुप में दिया गया। इसके उपरांत पानी से कुल्ला कराया गया। अगले ही दिन यह प्रक्रिया काली चाय के काढ़े (१.५%) के साथ की गई। बरकरार खाद्य कणों मे सलाईवरी एमाईलेज गतिविधि माल्टोज सूक्रोज की मात्रा से क्रोमैटोग्राफी के माध्यम निर्धारित की गयी। जबकि दोनों समुहों की तुलना सांख्यिकीय रूप से महत्त्वपूर्ण नहीं थी, माल्टोज सूक्रोज का औसत अनुपात प्रतिशत कमी उच्च क्षय समूह में करने के लिए ४३.६३% और अल्प क्षय समूह में ४१.१७% है जो अत्यधिक महत्त्वपूर्ण (पी₹०.००५) था। चाय के काढ़े का सलाईवरी एमाईलेज गतिविधि पर दंत क्षय पर निरोधात्मक प्रभाव है, लेकिन उच्च और अल्प क्षय सूचकांक बच्चों में गतिविधि में कोई अंतर नहीं है।