

# Anticariogenic Activity of Black Tea - An In vivo Study

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## ABSTRACT

**Introduction:** Teas is known for its anticariogenic properties and various mechanisms have been invoked to explain this effect. One such proposed mechanism is inhibition of salivary alpha amylase activity by endogenous tannins present in tea.

**Aim:** The objective of the present study was to determine whether or not the ingestion of black tea decoction inhibits the enzyme salivary amylase and thus interferes with the release of maltose from intraoral entrapped particles of food.

**Materials and Methods:** A total of 30 children in the age group of 12 - 15 years were selected for the study. After two hours of fasting subjects consumed two salted crackers for 60 second following which they rinsed with water (control solution) and then with 1.5% black tea decoction (test solution) next day.

Retained food particles were recovered from buccal aspect of left mandibular premolar and salivary amylase activity was noted via chromatography. Paired t-test was applied for statistical analysis.

**Results:** Maltose to Sucrose ratio was used to evaluate the result. The average ratio was 3.27 for control solution and 1.82 for test solution. The results were statistically highly significant ( $p < 0.005$ ).

**Conclusion:** Tea inhibited the activity of salivary amylase and this inhibition assumes a special significance when it is considered that the effect of tea could be manifested over a prolonged period of time, as in a real life situation.

**Keywords:** Amylase, Maltose, Sucrose, Tannin

## INTRODUCTION

Dental caries is a ubiquitous, dietobacterial disease which still prevails as a worldwide epidemic and is a matter of critical concern even today. The concept of Key's triad for development of dental caries is universally accepted [1]. Three important components of this triad are susceptible host, cariogenic diet and bacteria. Multiple approaches have been adopted to prevent dental caries after taking these factors into consideration. Diet modification being one of them.

Teas is known for its anti-cariogenic properties & various mechanisms have been invoked to explain this effect [2-4]. One such mechanism of anti-cariogenic action of tea is inhibition of salivary alpha amylase activity by endogenous tannins present in tea [5,6]. Apparently this area holds future prospects as one of the preventive mechanisms from the vagaries of dental caries. So the present study was designed to evaluate the effect of ingestion of black tea decoction on the enzyme salivary amylase.

## AIM

The aim of the present study was to determine the effect of black tea decoction on salivary amylase activity in terms of release of maltose from intraoral entrapped particles of food in mouth.

## MATERIALS AND METHODS

The study was carried out at Nair Hospital Dental College in the Department of Pediatric and Preventive Dentistry. This was a clinical trial where participants were selected randomly and it was carried out for a duration of six months. The study was approved by the ethical committee of the institution and in accordance with Helsinki Declaration [7]. Necessary permissions were obtained from the Education Officer. School children were screened to evaluate their dental caries status. The caries status of children was assessed as per the WHO Criteria (1997) [8]. Children of 12 to 15 yrs age so as their permanent teeth are exposed to oral environment for sufficient time were included in the study. Children

with good general health were included in the study as activity of salivary amylase is affected by systemic diseases, certain drugs and microbial load [9]. Children with history of hospitalization or intake of antibiotics or medications in past six months were excluded from the study for same reason. The children who were medically and developmentally compromised were also excluded from study. A sample of 30 subjects fulfilling the criteria were selected for study randomly by lottery method. Sample size was calculated keeping in view at the most 5% risk, with minimum 80% power and 5% significance level (significant at 95% confidence level).

(**Formula:** Sample Size =  $n / [1 + (n/\text{population})]$ ).

In which  $n = Z^2 [P (1-P)/(D^2)]$ .

P = True proportion of factor in the population, or the expected frequency value (0.5).

D = Maximum difference between the sample mean and the population mean, (0.2 or 20%).

Or Expected Frequency Value minus (-) Worst Acceptable Value.

Z = Area under normal curve corresponding to the desired confidence level (1.96).

The calculation came out to be 24 but sample size taken was 30 for normality of the data. The procedure was explained in details to the respective parents or guardians and informed consent was obtained from them. The 1.5% tea decoction was used as test solution and water rinse as control.

## Preparation of tea decoction

Tea decoction was prepared by suspending 3gm of black tea powder (Taj Mahal brand) in 200ml of distilled water at 100°C, stirring gently for 3min, and filtering through Whatman filter paper no.1. A 1.5% solution of black tea was prepared. The estimation of tannins was done by Ragazzi and Veronese method via preparation of standard curves of gallic acid, estimation of total

phenolic content i.e., 1.56mg/ml and soluble phenolic content i.e. 0.59mg/ml [10].

### Collection of samples

Two hours of fasting period was a requirement, before the collection of food samples as activity of enzyme is initiated by eating starch. Each subject was given two salted crackers (2.8 g each with maltose to sucrose ratio of 0.4) to chew for approximately 60 seconds following which, they rinsed with 20ml water for 20 seconds. Retained food was recovered from buccal surface of left mandibular 1<sup>st</sup> or 2<sup>nd</sup> premolar after three minutes using curette and placed in 1ml distilled water in microcentrifuge tubes. The caps were closed and tubes were placed in boiling water for five minutes to deactivate amylase. The same subjects were called the next day because salivary amylase has diurnal pattern of secretion [9]. The same procedure was repeated using same amount of test solution i.e. 20ml tea decoction (1.5% solution with tannin concentration of 1.59 mg/ml) for standardization at room temperature. 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 1500g for five minutes and resultant supernatant fluid was diluted five folds with deionized water. Fifty micro liters aliquots of diluted

| Sr. No. | Maltose peak area unit calculated as area under the curve | Sucrose pea area unit calculated as area under the curve | Maltose to Sucrose ratio |
|---------|---|--|--------------------------|
| 1       | 5975  | 3937   | 1.52                     |
| 2       | 6741  | 3348   | 2.01                     |
| 3       | 1549  | 2942   | 0.53                     |
| 4       | 4245  | 3281   | 1.30                     |
| 5       | 6266  | 3470   | 1.80                     |
| 6       | 12508   | 3900   | 3.20                     |
| 7       | 5122  | 2883   | 1.78                     |
| 8       | 3263  | 3287   | 0.99                     |
| 9       | 10477   | 4234   | 2.47                     |
| 10      | 3046  | 1268   | 2.40                     |
| 11      | 8766  | 3875   | 2.26                     |
| 12      | 1987  | 2360   | 0.84                     |
| 13      | 3169  | 1648   | 1.92                     |
| 14      | 5376  | 3318   | 1.62                     |
| 15      | 27903   | 9833   | 2.84                     |
| 16      | 8223  | 8101   | 1.02                     |
| 17      | 1722  | 1048   | 1.69                     |
| 18      | 5753  | 3775   | 1.52                     |
| 19      | 3366  | 1799   | 1.87                     |
| 20      | 5811  | 3259   | 1.78                     |
| 21      | 1233  | 877  | 1.41                     |
| 22      | 3155  | 2127   | 1.48                     |
| 23      | 1720  | 627  | 2.74                     |
| 24      | 1524  | 1265   | 1.20                     |
| 25      | 6605  | 3136   | 2.11                     |
| 26      | 2849  | 1474   | 1.93                     |
| 27      | 6174  | 2466   | 2.50                     |
| 28      | 8007  | 4877   | 1.64                     |
| 29      | 10640   | 2363   | 2.21                     |
| 30      | 20163   | 8925   | 2.25                     |

**[Table/Fig-1]:** Maltose to sucrose ratios in children with tea rinses with average maltose /sucrose ratio 1.82.

samples were used for chromatographic analysis of maltose and sucrose using Dionex High Performance Anion Exchange Liquid Chromatography (HPLC) system, with Carbo Pac PA1 column. Sodium hydroxide was used as eluant. 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.

### 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 action of salivary amylase on starches. The threshold for the maltose to sucrose ratios of both the groups was assessed from chromatograms. Average maltose to sucrose ratio in test group i.e. after rinse with tea decoction was 1.82 [Table/Fig-1] and control i.e., after rinse with water was 3.27 [Table/Fig-2]. Student t test was used for comparison between two groups [Table/Fig-3,4]. Mean percentage reduction in maltose to sucrose was 42.40% and results were highly significant  $p < 0.005$  [Table/Fig-3].

| Sr. No. | Maltose concentration Peak area unit | Sucrose concentration Peak area unit | Maltose to Sucrose ratio |
|---------|--------------------------------------|--------------------------------------|--------------------------|
| 1       | 8027                                 | 3380                                 | 2.38                     |
| 2       | 14849                                | 4229                                 | 3.51                     |
| 3       | 2268                                 | 3081                                 | 0.74                     |
| 4       | 3043                                 | 1673                                 | 1.84                     |
| 5       | 15045                                | 4480                                 | 3.36                     |
| 6       | 23017                                | 4076                                 | 5.65                     |
| 7       | 14360                                | 3909                                 | 3.67                     |
| 8       | 3667                                 | 2158                                 | 1.70                     |
| 9       | 6807                                 | 1088                                 | 6.26                     |
| 10      | 11333                                | 2083                                 | 5.44                     |
| 11      | 6883                                 | 2087                                 | 3.01                     |
| 12      | 6327                                 | 4350                                 | 1.45                     |
| 13      | 2294                                 | 867                                  | 2.64                     |
| 14      | 8431                                 | 2823                                 | 2.99                     |
| 15      | 643                                  | 130                                  | 4.95                     |
| 16      | 10929                                | 3697                                 | 2.96                     |
| 17      | 5162                                 | 2148                                 | 2.40                     |
| 18      | 11952                                | 4308                                 | 2.78                     |
| 19      | 5919                                 | 1550                                 | 3.82                     |
| 20      | 7724                                 | 2605                                 | 2.97                     |
| 21      | 7978                                 | 2080                                 | 3.84                     |
| 22      | 3917                                 | 1752                                 | 2.24                     |
| 23      | 15309                                | 3571                                 | 4.29                     |
| 24      | 13057                                | 4553                                 | 2.87                     |
| 25      | 9076                                 | 2487                                 | 3.65                     |
| 26      | 8579                                 | 2984                                 | 2.88                     |
| 27      | 8560                                 | 2212                                 | 3.87                     |
| 28      | 11795                                | 4687                                 | 2.51                     |
| 29      | 8132                                 | 2363                                 | 3.44                     |
| 30      | 14112                                | 3242                                 | 4.35                     |

**[Table/Fig-2]:** Maltose to sucrose ratios children with water rinses with average maltose /sucrose ratio 3.27.

| Number of subjects | Mean percentage reduction in maltose/ sucrose | t     | Significance |
|--------------------|---|-------|--------------|
| 30                 | 42.4001                                       | 10.01 | <0.0001      |

**[Table/Fig-3]:** Applying student paired t test at 95% level of significance.

| Sr. No | Maltose to sucrose ratio with tea decoction | Maltose to sucrose ratio with water | Deviation= difference-mean difference | Standard deviation |
|--------|---|-------------------------------------|---------------------------------------|--------------------|
| 1      | 1.52  | 2.38                                | -0.59                                 | 0.35               |
| 2      | 2.01  | 3.51                                | 0.05                                  | 0.00               |
| 3      | 0.53  | 0.74                                | -1.24                                 | 1.55               |
| 4      | 1.30  | 1.84                                | -0.91                                 | 0.84               |
| 5      | 1.80  | 3.36                                | 0.11                                  | 0.01               |
| 6      | 3.20  | 5.65                                | 1.00                                  | 0.99               |
| 7      | 1.78  | 3.67                                | 0.44                                  | 0.19               |
| 8      | 0.99  | 1.70                                | -0.74                                 | 0.55               |
| 9      | 2.47  | 6.26                                | 2.34                                  | 5.46               |
| 10     | 2.40  | 5.44                                | 1.59                                  | 2.51               |
| 11     | 2.26  | 3.01                                | -0.70                                 | 0.50               |
| 12     | 0.84  | 1.45                                | -0.84                                 | 0.71               |
| 13     | 1.92  | 2.64                                | -0.73                                 | 0.54               |
| 14     | 1.62  | 2.99                                | -0.08                                 | 0.01               |
| 15     | 2.84  | 4.95                                | 0.66                                  | 0.43               |
| 16     | 1.02  | 2.96                                | 0.49                                  | 0.24               |
| 17     | 1.69  | 2.40                                | -0.74                                 | 0.55               |
| 18     | 1.52  | 2.78                                | -0.19                                 | 0.04               |
| 19     | 1.87  | 3.82                                | 0.50                                  | 0.25               |
| 20     | 1.78  | 2.97                                | -0.26                                 | 0.07               |
| 21     | 1.41  | 3.84                                | 0.98                                  | 0.95               |
| 22     | 1.48  | 2.25                                | -0.69                                 | 0.48               |
| 23     | 2.74  | 4.29                                | 0.10                                  | 0.01               |
| 24     | 1.20  | 2.87                                | 0.22                                  | 0.05               |
| 25     | 2.11  | 3.65                                | 0.09                                  | 0.01               |
| 26     | 1.93  | 2.88                                | -0.50                                 | 0.25               |
| 27     | 2.50  | 3.87                                | -0.08                                 | 0.01               |
| 28     | 1.64  | 2.51                                | -0.58                                 | 0.34               |
| 29     | 2.21  | 3.44                                | -0.22                                 | 0.05               |
| 30     | 2.25  | 4.35                                | 0.65                                  | 0.42               |

**[Table/Fig-4]:** Table showing deviation and standard deviation calculated.

## DISCUSSION

Starch products when combined with sucrose for sweetness have been found to be more cariogenic than either component alone [11,12]. Salivary alpha amylase plays an important role in oral fermentation of starch by catalyzing conversion of starch to maltose and maltotriose [13,14]. These reaction products are more diffusible than the large starch molecules, are acidogenic and have an intraoral demineralizing potential close to that of sucrose and glucose [15].

Numerous studies have demonstrated that polyphenols (the generic name for tannins) have the ability to bind proteins and thus inhibit enzymes [5,6,16,17]. Mormann and Muhlemann (1981) reported that the inhibition of salivary amylase can lead to a reduction in the incidence of caries in rats [18]. Thus we investigated how the activities of alpha-amylase are affected by polyphenols i.e., tannins present in black tea extract in vivo. Our results state that black tea decoction lead to statistically significant inhibitory effect on salivary amylase activity in mouth after short interval of consumption of starch rich diet which was in consensus with study done by Kashket S et al., [5].

Zhang J et al., studied effect of 1% tea decoctions were prepared and shown to inhibit salivary as well as bacterial amylase in vitro [6]. Further in-vivo intraoral experiments were carried out on six subjects to determine whether tea decoctions would interfere with the release of maltose in 'food particles that become entrapped on dentition. Maltose release was reduced up to 70% after

rinsing with tea. Samples were recovered at 0.5, 1 & 1.5 minutes. Black tea decoctions were significantly more effective than green tea decoction. Total polyphenol concentration of the black tea decoction used was 0.80 mg/ml with condensed polyphenol concentration being 0.24 mg/ml. As a comparison to the above study, in the present study 1.5% black tea decoction gave reduction of maltose release ranging from 30-60%. Samples were recovered after three minutes. Total polyphenol concentration was 1.56 mg/ml with condensable polyphenols being 0.59 mg/ml. The reason for lesser reduction at even higher concentration of black tea might be the delayed recovery of the food sample from mouth, during which the effect of tea might be reduced.

Hara Y, Honda M. reported that alpha-amylase can be inhibited by polyphenolic components of tea [16]. It was an in-vitro experiment in which four kinds of tea catechins and their isomers were tested for their salivary alpha-amylase inhibitory action. Alpha amylase activity was expressed in terms of moles of maltose liberated in one minute by one unit of alpha- amylase. The aflavin digallate was found to be most potent inhibitor of salivary amylase. The inhibitions were seen at concentrations of 0.5 mg - 120 mg/ml. A direct comparison of the present study with the above study is not possible. However in vitro data of the above study tends to support that polyphenols are inhibitors of salivary alpha-amylase.

Further comparison of effect of tea decoction between high caries and low caries rate children was not done in our study. Tea is known to be a source of dietary fluoride and it has been proposed that the anticariogenic effect of tea is due to the endogenous fluoride [19].

## LIMITATION

The limitation of our study was that sample size was very small, no comparison of level of fluoride and inhibition of salivary amylase was done in the present study.

## CONCLUSION

Salivary amylase is an important catalytic enzyme in digestion of starch. We studied the effect of black tea decoction (1.5%) on this enzyme in 30 children in age group of 12 to 15 yrs. There was significant inhibitory action of black tea decoction in comparison to test solution i.e., water rinses thus indicating the anticariogenic action of black tea. Thus use of black tea solution can be a significant and widely available measure in prevention of dental caries.

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