Camellia sinensis (Tea): Implications and role in preventing dental decay

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Submitted: 22-02-2013 Revised: 07-03-2013 Published: **-**-****

ABSTRACT

Tea is one of the most commonly consumed beverages with bioactive compounds like polyphenols-flavonoids-catechins, which are thought to be responsible for the health benefits that have traditionally been attributed to the tea. These compounds have multi-dimensional effects such as antibacterial action, inhibitory action on the bacterial and salivary amylase and inhibition of acid production. This article outlines the possible role of these compounds coupled with the presence of high fluoride content in tea to exhibit an anticariogenic effect.

Key words: Anti-cariogenicity, *camellia sinensis* (Tea), catechins, fluoride, health benefits

INTRODUCTION

Dental caries is the most widely spread disease of calcified tissues of teeth. The basic patho-physiology of the disease involves the demineralization of the inorganic component and the subsequent breakdown of the organic moieties of enamel and dentin. According to Miller, the etiology of dental caries has been associated with acid dissolution of the mineral tooth components, the acid being produced by oral bacteria using dietary carbohydrates as a substrate. In other words, dental caries is a multi-factorial disease which requires a susceptible host, a cariogenic microflora and a suitable substrate that must be present for a sufficient length of time.

To investigate the role of diet or substrate in the etiology of dental caries, a number of studies have been carried out until date. In 1967, Ernest Newbrun described sucrose as the "arch-criminal of dental caries".[1] Sucrose is utilized by the microflora to produce acid, which in turn causes dissolution of the tooth. In addition to this Sucrose can form glucan, which enables firm bacterial adhesion to teeth and reduces the diffusion of acid and buffers in the plaque. Replacement of sucrose with a less cariogenic or a non-cariogenic substrate has always been sought out as an effective method for the prevention of dental caries.

Moreover, there are certain food items, which are considered as caries protective or anticariogenic. These include milk, cheese, cocoa beans, unrefined plant foods, and tea.[2] Tea has been investigated in detail for its caries protective role and several studies have reported its efficacy on dental health.

Tea: Role in protection from dental caries

Tea is one of the most commonly consumed beverages in the world.[3] Tea is obtained from the dried leaves of plant *Camellia sinensis*. *C. sinensis* is an evergreen plant which can grow up to 25-30 feet, but in plantation they are clipped at a height of 2-3 feet. The shrub is heavily branched with dark-green, hairy, oblong, ovate leaves cultivated, and preferentially picked as young shoots. Based on the processing of the leaves three different types of tea are available, viz: Green tea, black tea, and oolong tea. Black tea is prepared by the curing process of maceration and oxidation by exposure to atmospheric oxygen.[3] To prepare green tea enzymatic oxidation is prevented by steaming the fresh leaves whereas, oolong tea is semi-fermented to permit a moderate level of enzymatic oxidation during processing.[4] Green tea is more popular in Japan and China whereas, black tea is consumed world-wide. The consumption of oolong tea is mostly restricted to China and Taiwan.[3]

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Access this article online

Quick Response Code:

Website: www.phcogrev.com

DOI: 10.4103/0973-7847.120515

Tea is reported to contain nearly 4000 bioactive compounds of which one third is contributed by polyphenols.[5] Other compounds are alkaloids (caffeine, theophylline and theobromine), amino acids, carbohydrates, proteins, chlorophyll, volatile organic compounds (chemicals that readily produce vapors and contribute to the odor of tea), fluoride, aluminum, minerals, and trace elements.[6] Polyphenols found in tea are mostly flavonoids.[7,8]

Research has shown that tea certainly possesses significant anti-cariogenic properties by virtue of the various anti streptococcal actions, which are discussed further in detail.

Anti-bacterial action

Tea specially, the green tea contains a significant amount of catechins. Catechins are a sub group of flavonoids and are thought to be responsible for the health benefits that have traditionally been attributed to the tea.[9] Major catechins are (−) epicatechin gallate (ECG), (−) epicatechin, (+) gallocatechin (GC), (−) epigallocatechin (EGC) and (−) epigallocatechin gallate (EGCG). GC, EGC and EGCG possess strong bactericidal as well as antibacterial activity.[10] Black tea contains much lower concentrations of these catechins than green tea.[11]

Although the mode of action of tea catechins is not exactly known, in order to explain the antibacterial action of EGCG, Ikigai *et al*. conducted a study in 1993 and concluded that high EGCG concentrations irreversibly damaged the bacterial cytoplasmic membrane, on the basis that phosphatidyl choline liposomes leaked fluorescent dye following exposure to the compound. However, the introduction of charged lipids into the liposomal bi-layer, reflecting more appropriately the composition of the cytoplasmic membranes of Gram-positive and Gram-negative bacteria, reduced the extent of this damage.[12] It has recently been claimed that cytoplasmic membrane damage results from the generation of hydrogen peroxide by EGCG within the bi-layer.[13]

Several workers have reported that catechins are inhibitory for Streptococcus mutans and Streptococcus sobrinus, with minimum inhibitory concentration ranging between 50 and 1000 μg/ml, well within the concentrations found in brewed tea.[10,14,15] A significant bactericidal effect was found after a brief exposure to 1 mg/ml of EGCG. (Kubo *et al*. 1992) found that many of the "flavor compounds" (e.g., Nerolidol) found in green tea, although present in too low a concentration to have a direct antibacterial effect, might act synergistically with the abundant catechins.[16]

Rasheed and Haider in their study separated different bacteria from saliva and teeth of cariogenic patients and identified by a variety of morphological and biochemical tests. Extracts of green tea strongly inhibited *Escherichia coli,* Streptococcus *salivarius and S. mutans*.[16] A major difference has been observed in the MIC and other antimicrobial parameters in the different studies reported. Xu *et al*.[17] found that the antimicrobial activity depended on the culture medium composition because medium proteins may bind to or even precipitate tea catechins, compromising their efficacy. This can explain the discrepancies among previous studies. Furthermore, many authors found that bacteria living in dental plaque are generally more resistant than culture bacteria normally used for the *in vitro* studies.[18,19]

Inhibition of salivary and bacterial amylase

Salivary amylases catalyze the hydrolytic cleavage of food starches to maltose and other low molecular weight sugars that are considered to be important in the development of dental caries.[20] Thus, early epidemiological studies indicated a positive correlation between dental caries and high salivary amylase activity,[21] although later studies have been less conclusive about the relationship.[22] The amylase enzyme is also produced by the oral bacterial microflora, which catalyses the breakdown of complex dietary sugars in to simpler low molecular weight moieties.

Tea beverage inhibits salivary amylase, under conditions existing in the mouth during and shortly after consumption of tea drink.[23] It has been shown that brews of several black and green teas also suppressed amylase activity from *S. mutans*. In contrast to other anti-cariogenic actions black teas were more active against both types of enzyme[24] than the green tea. This finding was interpreted as suggesting that higher molecular weight polyphenols (more abundant in black tea) were responsible for the anti-enzymatic activity. Not only catechins but also theaflavins (present only in black tea) inhibited salivary amylase.[25]

A study was carried out in which effects of purified tannic acid, catechin and fluoride on salivary amylase activity were determined. Inhibition was greatest with tannic acid while catechin was inhibitory only at concentrations greater than 2 mg/ml. Fluoride, in the range from 0.1 ug/ml to 10 ug/ml, did not show any inhibition of the enzyme. Generally, black teas were more effective than green teas, consistent with the lower levels of condensed tannins in the latter. Indeed, the correlation between the levels of enzyme inhibition and tannin content was high and was supported by the finding that inhibition was completely lost after precipitation of the tannins with gelatin. Apparently, the major inhibitors in the different teas were the tannins. Catechin was effective in this experiment as well, but it is important to note inhibition was observed only at concentrations above 2 mg/ml. Even at these concentrations, catechin was not a potent inhibitor (13.2% inhibition at 3.3 mg/ml).[24]

Snacks frequently consumed with tea tend to be retained on the dentition for relatively long periods of time where they act as slow-release sources of fermentable carbohydrate.[26] Results from the intra-oral studies showed that tea inhibited

the activity of salivary and perhaps, bacterial amylases that became admixed with the salted crackers (snacks) during chewing. In other words, the inhibitory constituents in the teas were able to penetrate quite readily into the masses of food that were retained on the dentition. The consequence of amylase inhibition assumes a special significance when it is considered that the effect of the tea could be manifested over a prolonged period of time.

Inhibition of acid production by tea

Microorganisms like *S. mutans* ferment carbohydrates present in the diet and produces acid as the byproduct. This acid causes demineralization of tooth leading to carious lesions. Certain studies have shown an inhibitory effect of tea over the acid production by cariogenic bacteria thus imparting a caries protective action.

Hirasawa *et al*.[27] investigated the effects of EGCG on the bactericidal activity and acid production from plaque bacteria and *S. mutans* in detail. Further in this study, the inhibitory effect of individual tea catechins extracted from green tea on the activity of lactate dehydrogenase (LDH), which converts pyruvic acid to lactic acid was also examined. In this experiment, the subjects were asked to rinse with EGCG of different concentrations at different intervals. It was found that more inhibition was observed after a 30 min interval between rinses than after 2 min or 120 min intervals. The minimum pH after the 30 min interval was 6.5, compared to 4.8 in the water rinse control.

The most effective concentration of EGCG was 5 mg/ml, but 2 mg/ml EGCG yielded a similar pH time course to 5 mg/ml. As the 5 mg/ml solution of EGCG was too bitter for daily use, 2 mg/ml concentration was used for further experiments. The inhibition of plaque pH fall by EGCG after too short (2 min) or too long (120 min) an interval was not satisfactory. This finding suggests that the reaction between EGCG and dental plaque needs a suitable interval for penetration of EGCG into the dental plaque. To be effective, more than 2 mg/ml of EGCG is required for mouth-rinsing. EGCG solutions stronger than 5 mg/ml tasted bitter and thus would not be appropriate for daily use. The major catechin in Japanese green tea is EGCG and typical preparations of green tea contain approximately 0.5-1.0 mg/ml catechins. Thus, regular drinking of green tea might effectively inhibit these bacteria. The study suggested that the application of catechins on a daily basis may be a useful method of preventing dental caries.

The interval until reaching the critical pH when the bacteria had been pre-incubated with EGCG was two to three times longer than that without catechin pretreatment for both *S. mutans* and *S. sobrinus*. It was also found that the percent inhibition of LDH activity caused by EGCG, ECG and catechin complex was 99, 92 and 80% respectively.

Awadalla *et al*. conducted a clinical pilot study on 25 human subjects in year 2011. The researchers recorded S. mutans count in saliva and plaque, Salivary and plaque pH values and Gingival Bleeding Index before and after rinsing with green tea for 5 min. This study supports the effectiveness of local application of green tea as antibacterial and anticariogenic material as it decreases the acidity of saliva and plaque.[28]

Xu *et al*.[17] in their detailed *in vitro* study determined the effect of EGCG on acid production by *S. mutans* by monitoring the glycolytic pH drop of *S. mutans* culture. The acid production by *S. mutans* cells was significantly inhibited by EGCG at sub-MIC levels. The inhibitory effect on acid production may result from the biological effect of EGCG on the bacterial glycolytic pathways. Not only may the suppression of enolase by EGCG at the transcriptional level exert direct inhibition on glycolysis, but also the reduction of downstream phosphoenolpyruvate (PEP) produced by enolase may suppress the PEP: Carbohydrate phosphotransferase system, the major system for internalizing sugar into the cell during a depleted sugar situation.[29] The net result would be a decrease in sugar internalization and suppressed glycolysis, leading to the reduced acid production by S. mutans cells as observed by Xu *et al*. in their study.

Role of fluoride present in tea in preventing caries

Ramsey *et al*. reported 35 years ago that there was a significant inverse correlation in children between amounts of tea drunk daily and DMFT score; they attributed this at the time to an increased fluoride intake.[30] Tea contains a very high quantity of fluorides in it.

Suyama *et al*. conducted an *in vivo* study to evaluate enamel re-mineralization and the acquisition of acid resistance by using the sugar-free chewing gum containing fluoride extracted from green tea. In this study, 45 volunteers participated in a crossover, double-blind study and wore intraoral appliances with the human demineralized enamel. Subjects chewed fluoride chewing gum (50 μg fluoride) or placebo gum. The results showed that fluoride containing chewing gum produced a superior level of remineralization and acid resistance as compared to the placebo gum.[31]

Although fluoride is a known potent caries protective agent; however, most of the reports suggest that the anti-caries effect observed with green tea is due primarily to the antibacterial properties of the organic components (polyphenols, tannins) rather than the cariostatic effect of fluoride.[32-34]

Alturfan *et al*. highlighted the safety concerns regarding the exposure to a higher level of fluorides in regular tea consumers. In their study, level of fluoride was analyzed in black tea, herbal and fruit infusions from various countries such as Turkey, Sri Lanka, India, and Kenya using an ion specific electrode. The content of fluoride in black tea extracts ranged from 0.57 mg/l to 3.72 mg/l after 5 min of

brewing. Higher fluoride levels were found in black teas originated from Turkey when compared to teas originated from Sri Lanka. The authors suggested indicating the fluoride concentration and daily safety precautions on tea products in countries like Turkey where tea is traditionally consumed.[35]

HUMAN STUDIES

Numerous *in-vitro* studies and studies using the animal models have been carried out to describe the anticariogenic potential of tea extracts. Unsurprisingly very few human trials have been carried out and none of them have concluded with a definitive outcome.[36] Elvin-Lewis and Steelman found significantly lower DMFT and plaque scores in children who habitually drank one-to-three cups of tea per day than in those whose intake was only one-to-two cusp per week.[37] Another study in the UK concluded that 14-year-old children who had tea (whether with added sugar or not) had a significantly lower DMFT score than coffee drinkers.[38] Koyama *et al*. analyzed cross sectional data from Ohsaki Cohort 2006 Study (Japan). They used multivariate logistic regression analysis to calculate odds ratios for tooth loss using 3 cut-off points of 10, 20, and 25 teeth relative to quantity of green tea consumption. The findings of the study indicated an association of green tea consumption with decreased odds for tooth loss.[39]

Ferrazzano *et al*. tested the effectiveness of an experimental green tea extract in reducing levels of *mutans streptococci* and *lactobacilli* in saliva. For this study 66 healthy individuals ranging in age from 12 years to 18 years were recruited and randomly divided into two groups. Group A subjects were asked to rinse their mouths with 40 ml of an experimental green tea extract, for 1 min, 3 times a day for a week, whereas Group B subjects were asked to rinse with 40 ml of a placebo mouth rinse. Saliva samples were obtained at baseline, 4 days, and 7 days. The counts of *mutans streptococci* and *lactobacilli* were investigated by chair-side kits and data were statistically processed. The result has shown that 60% of subjects using a green tea mouth rinse presented a significant lowering of levels of *S. mutans* and 42.4% of subjects using this green tea mouth rinse presented a significant lowering of levels of *lactobacilli* compared to the subjects using the placebo mouth rinse. Based on the results the authors suggested the daily use of a mouthwash of green tea infusion for the reduction of salivary levels of *mutans* and *lactobacilli*, which are the most virulent cariogenic pathogens in the oral cavity.[40]

CONCLUSION

The existing literature which supports the anticariogenic potential of tea is based mostly on animal and *in vitro* studies in abundance, but lack of sufficient clinical studies using human subjects limit the viability and acceptability of this compound as an active ingredient in prevention of dental decay. Tea "can be considered a functional food for oral health by controlling, through prevention, the most prevalent infectious disease of mankind: Caries."[41] This paper highlights the fact that the incorporation of tea extract in dental products such as dentifrices, mouthwash, dental floss, and chewing gums can prove to be helpful in prevention of dental caries thus, expanding its horizon from academic to clinical set-up.

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How to cite this Article: Goenka P, Sarawgi A, Karun V, Nigam AG, Dutta S, Marwah N. *Camellia sinensis* (Tea): Implications and role in preventing dental decay. Phcog Rev 2013;7:152-6.

Source of Support: Nil, **Conflict of Interest:** None declared