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Is black tea (Camellia sinensis) bacteriostatic against *Streptococcus mutans*?

An in vitro research report.

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Abstract

Background: *Camelia sinensis*, tea is associated with reduction of caries. Tooth decay occurs from microbes attacking decalcified tooth niches. Among bacteria responsible for decay is *Streptococcus*. Reports of effects of tea on Streptococcus are rare. **Aim:** This research reports *in vitro* modulation of tea, with common additives (sugar and lemon), on Streptococcal growth. ***Methodology***: Concentrations (10^2 to10^7 CFU/mL) *Streptococcus mutans* were prepared in various growth media. Bacterial mulitplication was measured by light absorbance. ***Test media were:*** **A-** Black-tea; **B-** Black-tea with lemon juice; **C-** Black-tea with sugar; **D-** Black-tea with lemon juice and sugar; **E-** Purified Water. The pH of each liquid was measured to start. Test liquids were inoculated and incubated for 24 hrs at 37C^o. Absorbance by time per microorganism concentration per solution was measured. Absorbance measured at 625 nm at time intervals 0 min, 5, 15, 20, 30 , and 60 minutes per solution **(A to E)** and per microorganism concentration (10^2 to 10^7 CFU/mL). ***Results:*** pH's of the different test solutions range from pH 3.68 (black tea with lemon juice and sugar) to 5.49 (plain black Tea); pH of controls were pH 6.01 (purified water) and pH 7.01 (Ringer solution). ***Conclusion:*** Black-tea inhibits *S. mutans* growth in the first15-30 minutes. Black-tea with lemon, enhances this bacterial inhibition. Adding sucrose accelerates bacterial growth. These results show that black tea plays a role in inhibition of decay formation, but adding sucrose accelerates bacterial growth. Abbreviations used in this article:

PBUF =phosphate buffer

IRB= Internal Review Board

S. = Streptococcus

TSA = tryptic soy agar

TSB =tryptic soy broth

CFU=Colony Forming Units

Keywords: social support, social networks, chronic illness, older adults, aged, elderly, self-management, and self-care

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Introduction:

Many beverages are consumed world-wide among which are alcoholic drinks like beers and wines, and non-alcoholic beverages like coffees and teas. When plain water is excluded, tea-water infusions made from leaves of the plant Camellia sinensis, is the most frequently, commonlyand widely consumed liquid beverage worldwide. Infusions of Camelia sinensis is consumed globally, as black tea. (1) Among health benefits derived from tea, is its effect on teeth. (2) Animal experimental research notes that black tea is cariostatic, (3,4) and epidemiologically black tea infusions are causally associated with reduction of prevalence of dental cavities in humans (6,7). Tea is known to contain many micronutrients like, Sodium, Potassium, Aluminium, Copper, Zinc and Fluoride (8,9) Thecariostatic effect is ascribed to the influence of fluorides, polyphenols and cathechins in tea interfering with the decalcification and bacterial cavitation processes. (10). Gnotobiotic animals do not develop tooth decay.(11) Tooth decay occurs from stagnating intra-oral biofilm bacteria attacking vulnerable decalcified tooth niches.(12) Although biofilms vary in microbial composition, depending on the duration of stagnation, dental caries initiation depends on a bacteria producing an extracellular matrix of polysaccharide [an exo-polymer], changing the biofilm acidity to below critical pH5.5and bacteria causing destruction as a cavityinto the decalcified locus. (13)It has been known for decades that among the major cariogenic species in oral biofilm responsible for dental decay is Streptococcus. Althoughmany varieties of Streptococci,[like Streptococcus mutans, S. faecalis, S. liquefaciens, S. mitis, S. pyogenes, S. aureus, S. sanguis and S. salivarius,]may be involved in the caries process, Streptococcus mutansremains the major and most commonly implicated. (14) This is because S.mutans synthesizes an extracellular exo-polysaccharide matrix, which acts as a dynamic, ionic exchange gradient, and allows acid decalcification of tooth material and subsequent bacterial attack. (13,14) Reports of tea affecting growth of whole biofilm exist (15) but reports on the direct effect of tea on growth of specific oral bacteria involved, S. mutans, in the caries process, are rare.

Aim:This research reports onin vitro modulation of tea, with common additives (sugar and lemon), on growth of ubiquitous oral Streptococci, namely S. mutans.

Materials and Methods:

The experimental procedure consisted of :

I. Preparing a concentrated suspension of S. mutans.

II. Determining the suspension concentration of each microorganism.

III. Seeding the microbes into preparation of tea.

IV. Measuring the growth of microbes in the different liquids.

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1- Prepare the microbial suspensions: grow the microorganism in TSB, incubate 24h at 30-35oC.

2- To determine the concentration:

a- Label 10 tubes (10-1 to 10-10) with 9mL of PBUF.

b- Transfer 1 mL of pure suspension from TSB to the first tube and prepare serial dilutions.

c- Plate 1mL in TSA in duplicate from each dilution.

d- Incubate the plates for 24h at 30-35oC,

e- Count the colonies on each plate.

f- Multiply the number of colonies count by the dilution factor.

3- Choose a serial concentration of 102 to 107 CFU/mL for each microorganism.

4- Take 2mL of each concentration, mix in 18 mL of Ringer solution.

5- Incubate 6 tubes having a mix concentration of each microorganism from 102 to 107 at 37oC for 24h.

6- Prepare the following solutions using purified water (see table 1 in article):

A- Regular Black tea

B- Black tea with lemon

C- Black tea with sugar

D- Black tea with lemon juice and sugar

E- Purified Water

7- Measure the pH for each solution (see table 1 in article).

8- Prepare 7 tubes having 18 mL of each solution (From A to E); add 2 mL of each microorganism suspension from step #5 to each solution (1 tube will not be inoculated with microorganism, using as negative control or Blank).

9- Incubate all tubes at 37oC immediately after inoculation.

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10- Samples from each solution were tested immediately after inoculation (before

incubation) and absorbance was measured using Genesys 20 Thermo Spectronic spectrophotometer (WL 625 nm), measuring at following time intervals: 5 min, 15 min, 30 min, 60 min. Readings were recorded by two technicians, the second unaware

of the aim of the experiment.

Five different solutions were used to test the growth of the bacteria.

Composition of solution used: n=7 with for 18 mL of each solution. The results presented are the means of readings from the 7 samples. ± Standard deviations were negligible and consequently ranges are not supplied.

Solution	Concentration	pH
A-Plain black tea	1 bag /175mL purified water	5.49
B-Black teat with lemon juice (citric acid)	1 bag /175mL purified water + 1% lemon juice (1.75mL)	3.72
C-Black tea with sugar	1 bag /175mL purified water + 2% sugar (3.5g)	4.92
D-Black tea with lemon juice (citric acid) and sugar	1 bag /175mL purified water + 1% lemon juice (1.75mL) + 2% sugar (3.5g)	3.68
E-Purified water	175mL purified water	6.01
Ringer Solution	25%	7.01

Table 1: Composition, concentration and pH per solution used. Color codes follow the graphs below.

Absorbances were measured at 625 nm at following time intervals 0 min, 5 min, 15 min, 20min, 30 min, and 60 min per solution (A to E) and per microorganism concentration (102 to 107 CFU/mL).

Results: All the data were used to estimate growth or inhibition of bacteria in

each solution. Below are graphs derived from numerical data.Figures 1 and 2 represent absorbance over 20 minutes for the same bacteria concentration in the different solutions.

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Although prolonged data were collected, the derived graphs reflect the first 20 minutes of the experiment. And figures 3, 4, and 5represent absorbance over 20 minutes for the same solutionsaveraged from different bacterial concentration.

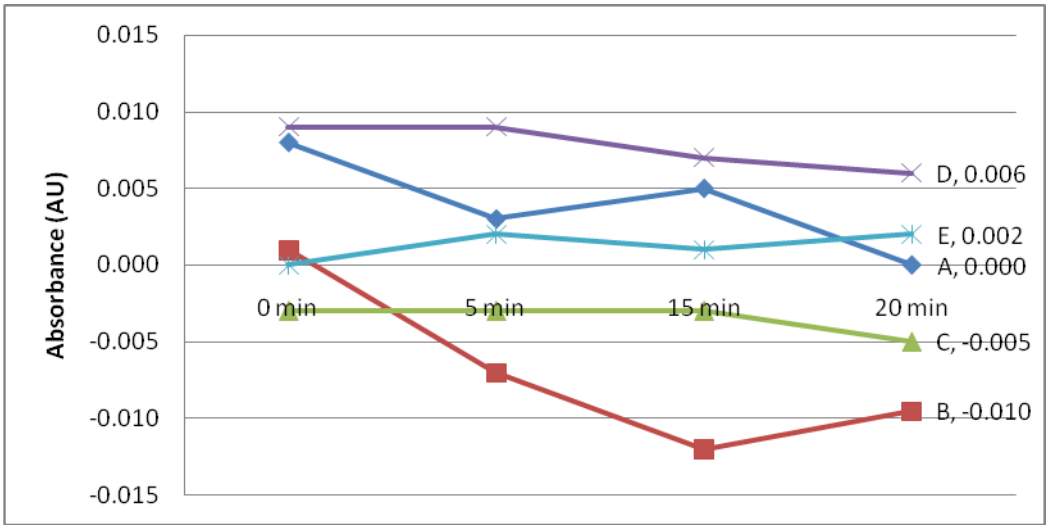

Figure 1: Absorbance graph over 20 minutes in each solution, at 10^6CFU/mL.A= Regular Black tea B= Black tea with lemon juice; C= Black tea with sugar D= Black tea with lemon juice and sugar E= Purified Water. The regular black tea (A) shows significantly ($p<0.05$, student-t) less absorbance than the control E and D reflecting inhibition of growth

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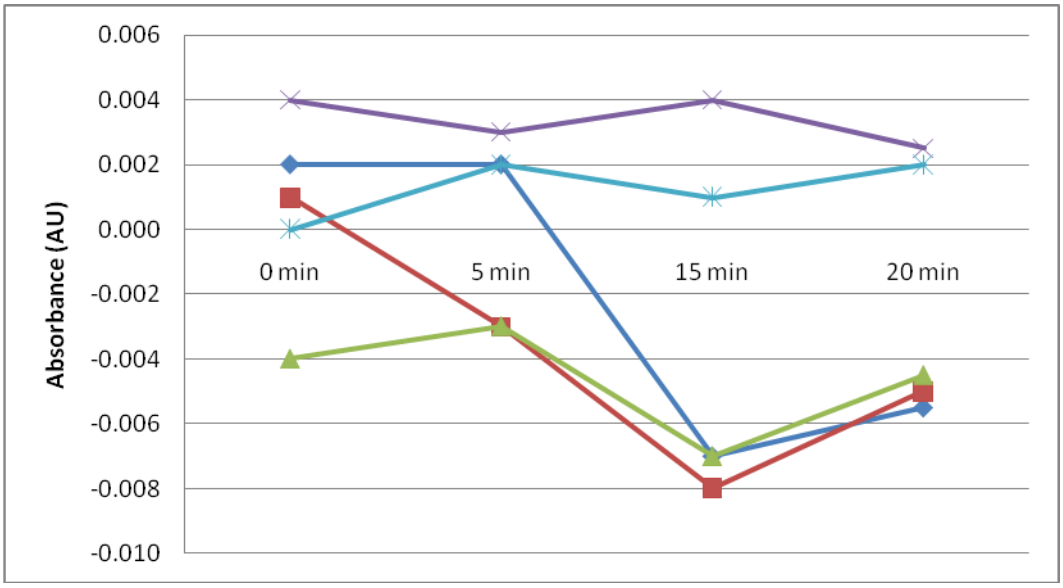

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Figure 2: Absorbance graph over 60 minutes in each solution, at 10^2CFU/mL.A= Regular Black tea B= Black tea with lemon juice; C= Black tea with sugar D= Black tea with lemon juice and sugar E= Purified Water. The regular black tea (A) shows significantly ($p<0.05$, student-t) less absorbance than the control E and D reflecting inhibition of growth.

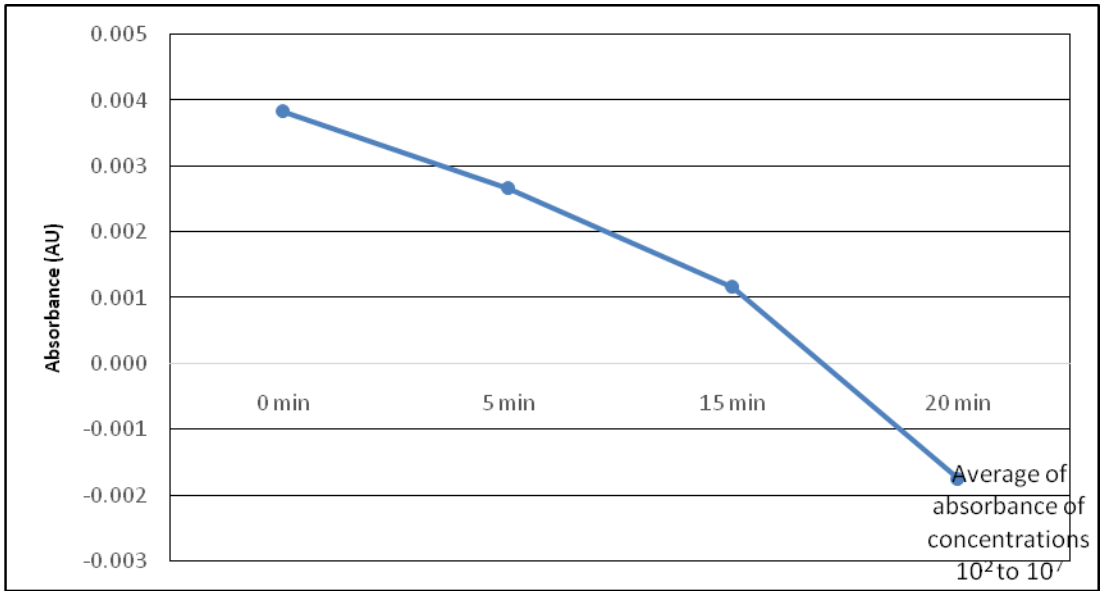

Figure 3: Absorbance graph of solution A over 20 minutes at different bacteria concentration (10^2-10^7CFU/mL). The regular black tea (this solution) shows significantly ($p<0.05$, student-t) less absorbance between at 20 min than min 0 reflecting inhibition of growth over time.

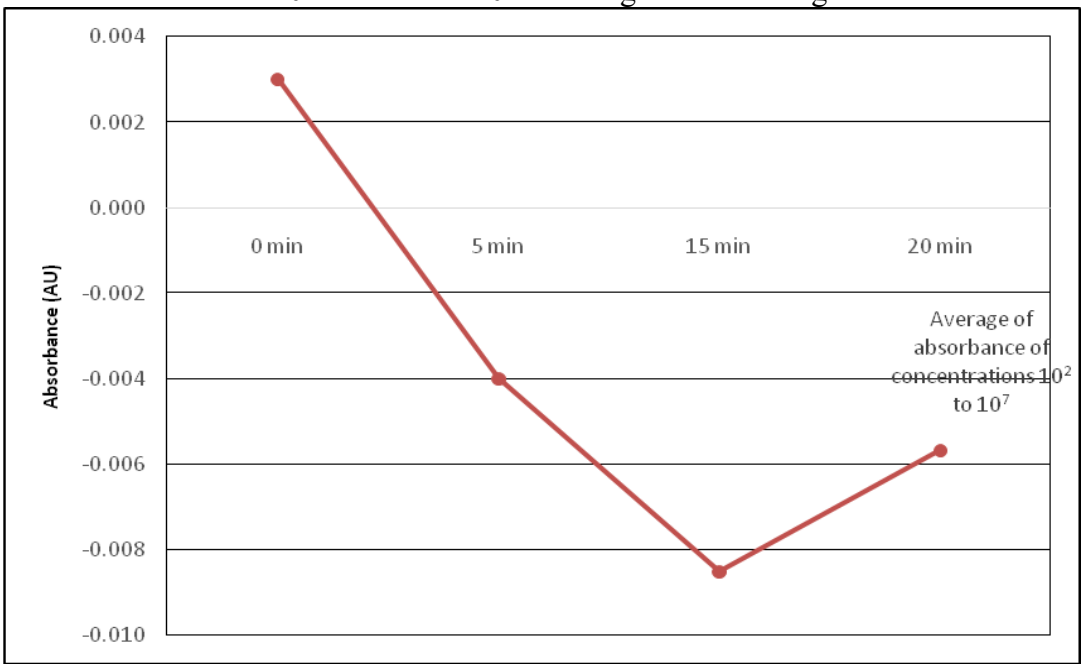

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Figure 4: Absorbance graph of solution B over 20 minutes at different bacteria concentration (10^2-10^7CFU/mL).The black tea with citric acid (this solution) shows significantly ($p<0.05$, student-t) less absorbance between at 20 min than min 0 reflecting inhibition of growth over time.

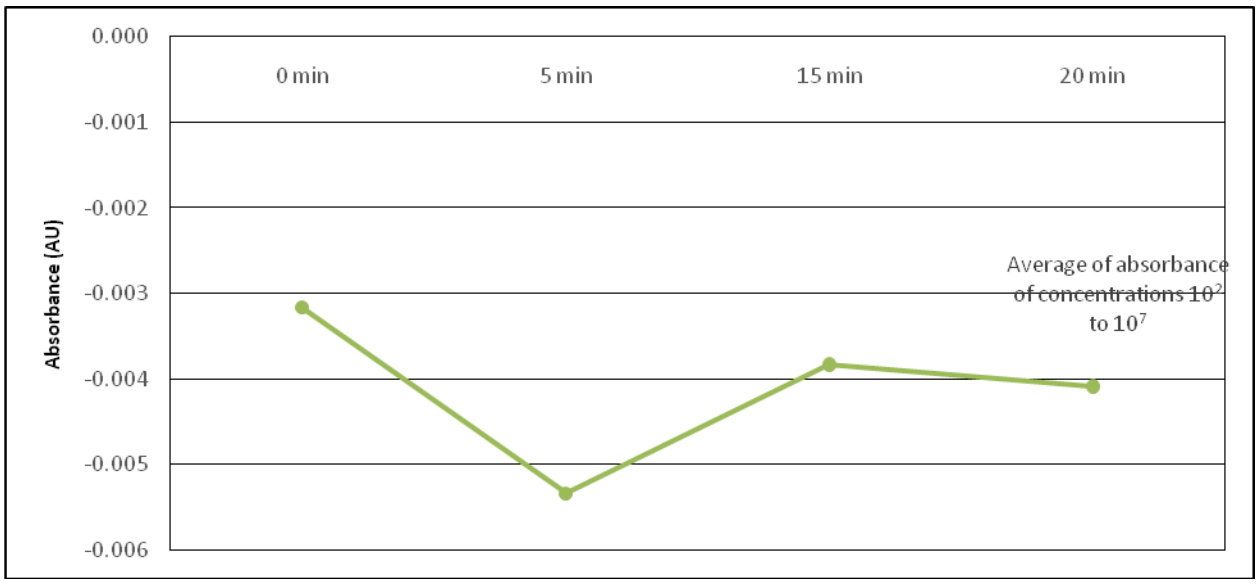

Figure 5: Absorbance graph of solution C over 20 minutes at different bacteria concentration (102-107CFU/mL).Black tea with sugar (this solution) does not show significant reduction in absorbance between 0 min and 20 min.

Statistics: The bacterial growth is significantly lower in the test liquid with plain black tea. Line A in figures 1 and 2 above. Tea with lemon juice has the most inhibitory effect. Line B in Figures 1 and 2 above. Sugar added to the tea promoted bacterial growth, with or without lemon juice. Line C and D in Figures 1 and 2 above. Whether at dilute (102) or high(106) bacterial counts, the results follow a similar pattern Fig 3, 4&5.

Discussion: Extrapolating from figures 1and2, high concentrations 106 bacteria [as is found in sessile biofilm], and low concentration 102 bacteria [as is found in planktonic colonies] both show inhibition of growth by tea. But the planktonic inhibition is more marked. To eliminate inter- and intra-operator error, all readings and results were checked by a second operator, unaware of the aims of the experiment. The water control (E-light blue in Figures 1 and 2) showed virtually no growth. Because of stimulated-saliva-flow, a bolus introduced into the mouth has been shown to be nearly completely eliminated through dilution and swallowing in less than half an hour, typically 20-25 minutes after ingestion.(16) Intra-oral pH measurements show that drinks with acidogenic carbohydrates (glucose, sucrose) will be cleared by stimulated

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saliva in 25 minutes (17). Accordingly, focus is directed primarily to what occurs in the first initial 30 minutes and less on the subsequent times.

In this experiment, CFUs at 107 to 102 all showed similar results with significant inhibition of bacterial growth; accordingly all the absorbances were decreased reflecting that Streptococcusmutans, and probably other gram positive similar acidogenicStreptococcalorganisms, are significantly inhibited by Camelia sinensis (black tea). This may be due to catechins or its known fluoride content.

Fluoride in foods, like fish, and beverages, likeCamellia sinensis tea infusions, are renown and recommended as natural sources of fluoride. Fluoride in water, between 1ppm and 0.7ppm is recommended as a safe form of reducing the prevalence of decay.(18,19, 20) The caries reduction reported in epidemiological studies with tea, is attributed to the effect ofthese dietary sources of fluoride.(5-7) The evidence from the experiments reported here indicates black tea inhibits the growth of Streptococci in vitro, and Black tea with lemon significantly inhibits Streptococcal growth more in vitro, than does black tea alone.(See graphs above). The acidity of the lemon juice, deriving from citric acid, modifies the pH and seems to augment the inhibitory effect of the black tea on the bacterial growth. Streptococci have enzymes (like glucosyl transferases), which voraciously metabolize fermentable carbohydrates, and increases in growth when sugar wasadded, were not unexpected. Black tea probably also inhibits early Streptococcal growth in vivo, and theevidence reported here provides more proof that the main bacterial species involved in dental decay, namely Streptococcus mutans, is inhibited by the black tea.

Bacterial counts in thick mature oral biofilms is 106 per mg this is challenging to replicate in vitro and for this reason different concentrations of CFUs were used in this experiment. Figure 3 clearly shows a significant trend that tea inhibits growth of S. mutans at the various CFU concentrations used.

Clinical implications: Humans consume at least one-and –a-half Liters(1.5 L)of water per day to sustainhealth, and many drink tea as part of their daily liquid intake.From the evidence presented here, imbibing black tea clearly shows drinking black tea inhibits growth of Strep mutans, and the consequent incidence of caries. This evidence supports dietary advice to substitute tea for sugary drinks, as tea is also very low in calories from fermentable carbohydrates. By regularly drinking black tea, not only will caries formation be reduced. also control of weight-gain is possible.

Concluding remarks: Black tea alone in vitro inhibits growth of Streptococcus mutans. When lemon is added this effect is enhanced. Adding sugar to tea inhibits this effect. The same influences probably occur in vivo.

Author's statement: The authors have no conflict of interest to declare. This was a spontaneous research by the authors and no funding was solicited or obtained.

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Ethics: Because no living animals or humans were involved, no IRB certificate was needed for this research.

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