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Original Article

Influence of black tea on *Streptococcus mutans* and *Lactobacillus* levels in saliva in a Saudi cohort

AbdelRahman M. Ramadan, PhD [a], Hala A. Bakeer, PhD [b], Mohamed S. Mahrous, PhD [c] and Tamer M. Hifnawy, PhD [c,*]

[a] *Periodontology and Oral Medicine, Preventive Dental Sciences Department Division of Periodontology, College of Dentistry, Taibah University, KSA*
[b] *Periodontology and Oral Medicine, Preventive Dental Sciences Department Division of Dental Public Health, College of Dentistry, Taibah University, KSA*
[c] *Medical Education Department, College of Dentistry, Taibah University, Madina, KSA*

الملخص

أهداف البحث: يرتبط تسوس الأسنان مع ارتفاع عدد المستعمرات من المكورات العقدية الطافرة والملبنة وانخفاض قدرة اللعاب على تخفيف تركيز هذه المستعمرات. تهدف هذه الدراسة لتقييم النشاط المضاد للميكروبات للشاي الأسود على البكتيريا المسببة لتسوس الأسنان من المكورات العقدية الطافرة والملبنة. وتم قياس النشاط المضاد للميكروبات بعدد وحدات تشكيل المستعمرات. تمت دراسة العلاقة بين هذه العوامل قبل تناول الشاي وبعد ساعة وساعتين من شرب الشاي في مجموعة من البالغين في المدينة المنورة.

طرق البحث: أجريت دراسة تجريبية مستقبلية متعددة مراحل المتابعة لتقييم النشاط المضاد للميكروبات للشاي الأسود على البكتيريا المسببة لتسوس الأسنان من المكورات العقدية الطافرة والملبنة. تم الحصول على عينات اللعاب بدون أي محفزات من المشاركين (قبل وبعد شرب الشاي على الفور بعد ساعة واحدة) عن طريق البصق إلى أوعية معقمة. وأخذت العينات إلى المختبر مباشرة من أجل وضعها في محضن لمعادلة درجة الحرارة ثم العد للمستعمرات المتكونة لاحقا. تم حساب أعداد للمكورات العقدية الطافرة والملبنة من اللعاب باستخدام اختبار قياس مخاطر التسوس.

النتائج: أجري البحث على ٢١ مشاركا، ١٣ من الذكور و٨ من الإناث الذين كان متوسط أعمارهم ٣٢.٦ ± ٨.٠٢. لم يكن للشاي الأسود تأثير معنوي على تقليل التعداد البكتيري.

الاستنتاجات: يمكن استنتاج أن الشاي الأسود أظهر تأثيرا مضادا للميكروبات ضئيل ضد المكورات العقدية الطافرة والملبنة.

الكلمات المفتاحية: الشاي الأسود؛ البكتيريا المسببة للتسوس؛ المكورات العقدية الطافرة؛ الملبنة

Abstract

Objectives: Dental caries are associated with high counts of S*treptococcus mutans* (SM) and *Lactobacillus* (LB) and low saliva buffering capacity (BC). This study aimed to evaluate the antimicrobial activity of black tea on salivary cariogenic microflora, SM and LB species in an adult population. Antimicrobial activity was measured from the number of colony forming units (CFUs) of SM and LB, and BC of saliva.

Methods: In this prospective experimental study, unstimulated saliva samples were acquired from the participants before, immediately after, and 1 h after drinking tea by collecting saliva in sterilised containers. Samples were taken to the laboratory for incubation and subsequent counting. SM and LB counts and BC of saliva were calculated using the caries risk test (CRT).

Results: A total of 21 participants, 13 males and 8 females, with a mean age of 32.6 ± 8.02, were recruited in this study. Black tea had no significant effect on reducing the cariogenic bacterial counts ($p > 0.05$).

* Corresponding address: Medical Education Department, College of Dentistry, Taibah University, Madina, KSA.
E-mail: thifnawy@taibahu.edu.sa (T.M. Hifnawy)

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Conclusion: Based on this study, it can be deduced that black tea exhibits an insignificant antimicrobial effect against *Streptococcus mutans* and *Lactobacillus* bacteria.

Introduction

Tea is one of the most popular beverages worldwide. Annually, 2.5 million metric tons of dried tea, 78% of which is processed as black tea, is produced and consumed. The annual tea consumption worldwide from 2013 to 2021. In 2018, global consumption of tea amounted to about 273 billion litres, and it is forecasted to reach 297 billion litres by 2021.[1,2] The beverage is made by soaking treated, dried *Camellia sinensis* leaves in hot water,[3] and it has been estimated that approximately 18−20 billion cups of tea are globally consumed daily.[4]

Depending on the treatment of *C. sinensis* leaves, three distinctive categories of tea are recognised: unfermented tea (green tea), semi-fermented tea (oolong tea), and fermented tea (black tea).[5] In general, the treatment of *C. sinensis* involves different types and degrees of oxidation, halting the oxidation, and formation and exposure to atmospheric oxygen for drying the tea leaves.[6]

Almost 4000 bioactive compounds in the form of polyphenols, flavonoids, and catechins are credited with health benefits of drinking tea.[7,8] Among these, polyphenols constitute the most interesting group and are the main bioactive molecules in tea. As a consequence, tea is deemed an important dietary source of polyphenols, principally the flavonoids.[9] Numerous other compounds are also associated with tea in the form of alkaloids (theobromine, theophylline, and caffeine), amino acids, chlorophyll, carbohydrates, proteins, and volatile organic compounds that bring out the natural flavour and aroma of the beverage. Furthermore, tea also contains trace amounts of fluoride and aluminium. Among these, the polyphenols constitute the most interesting group and form the main bioactive molecules in tea and, as a result, tea is deemed an important dietary source of polyphenols, principally the flavonoids.[10−12]

Some bioactive compounds in tea are attributed to effective bactericidal action against *Streptococcus mutans* and *Streptococcus sobrinus*. Tannic acid, found in tea polyphenols, is a significant inhibitor of bacterial growth, glucosyltransferase activity (thereby limiting the biosynthesis of adhesive glucan molecules), and both the human and bacterial amylases.[3,13,14] Furthermore, tea components have been shown to increase acid resistance of tooth enamel.[6,15]

S. mutans is an essential aetiological agent of dental caries, possesses numerous mechanisms to colonise tooth surfaces, and is speculated to play essential roles in the progression of caries.[16] *S. mutans* and *S. sobrinus,* which form the *Streptococci mutans* group, in addition to *Lactobacillus* species, are found in bacterial biofilms on tooth surfaces. These bacterial species are highly acidogenic in the presence of carbohydrates, are associated with the production of organic acids that cause enamel demineralisation,[17] and have been isolated from established carious lesions.[18]

The salivary levels of *S. mutans* and *Lactobacillus* have been associated with the number of decayed, filled, or missing teeth.[19−21] *Lactobacillus* spp. do not readily colonise tooth surfaces but may be transiently observed in the oral cavity even before tooth eruption. *Lactobacillus* spp. generally colonise the dorsum of the tongue and are found in the saliva due to the sloughing of the lingual epithelium.[22]

Various commercial chairside kits have been used to assess the levels of *S. mutans* and *Lactobacillus* and to evaluate their capability to predict dental caries. This study sought to measure the effect of black tea consumption on the levels of *S. mutans* and *Lactobacillus* in adults and the pH of the saliva over time.

Materials and Methods

The present study was a follow-up diagnostic clinical trial using a convenient sample. The study comprised of healthy female and male volunteers recruited from the administrative staff in the female and male sections of the College of Dentistry, Taibah University. The participants were recruited under the following inclusion criteria: dentate with at least 24 teeth present in the oral cavity, of general good health, and willingness to participate in the study procedures. The participants were excluded if they had undergone antibiotic treatment within the 14 days preceding the study or had used an antibacterial mouthwash during the 12 h prior to the study. They were also excluded if they had a fixed prosthesis, an orthodontic appliance, or conditions that influenced the examination procedure. After receiving both verbal and written explanations of the study protocol and its aims, participants signed a written informed consent written in both English and Arabic before the start of the study. Permission was obtained from Taibah University, College of Dentistry, Research Ethics Committee (TUCD-REC) with the approval number; TUC-DREC/05122013/Mahrous.

All participants received a clinical examination carried out in a dental chair, so that all examined participants were under the same lighting conditions, by one dentist. During the clinical examination, the oral health status of the participants and any decayed, filled, or missing teeth were recorded using the DMFT index.

The participants were directed not to eat, drink, chew gum, or wash their mouths for 2 h before the collection of their unstimulated whole saliva and until the test was

completed. The unstimulated whole saliva was collected in a private office to decrease any chance of stressful conditions. The saliva samples were obtained 2 h after breakfast, between 9 and 12 a.m. The saliva samples were collected in a dry sterilised plastic container to determine the *S. mutans* and the *Lactobacillus* counts and to measure the salivary pH.

Ivoclar Vivadent Caries Risk Test (CRT)[e] buffer was used to establish the salivary buffer capacity with a test strip, provided by the manufacturer, and their exclusive indicator system was used. The buffering capacity was limited to three levels, low, middle, or high, as provided by the manufacturer. The Ivoclar Vivadent CRT bacteria test kit permits the simultaneous determination of *S. mutans* and the *Lactobacillus* counts in saliva using selective agars. A film of foil was provided by the manufacturer to protect the agars from drying out or becoming contaminated during the procedure. The deep grooves in the agar carriers prevented the culture media from slipping out. Blue Mitis-Salivarius agar with bacitracin was utilised to determine *S. mutans* counts. Rogosa agar culture medium was utilised for to identify *Lactobacillus* counts.

According to the manufacturers' instructions, a sodium bicarbonate tablet ($NaHCO_3$) was added to each of the agar plates to release carbon dioxide on contact with moisture. This process creates favourable conditions to stimulate bacterial growth. The agar plates were then incubated at 37 °C for two days. After the 2-day incubation period, all colonies were counted. *S. mutans* was seen as small blue colonies on the blue agar while *Lactobacillus* spp. were seen as white colonies on the transparent agar. The findings were interpreted by reaching a consensus among the three investigators present at the time of evaluation using the provided model chart.

The number of colonies as calculated by the colony forming units (CFUs) per millilitre of saliva was a score given contingent on an observed number of CFUs existing against a model reference chart provided in the instruction manual provided by the manufacturer. Comparison with the matching pictures in the model chart allowed the assessment of dental caries risk. In the milieu, counts higher than 10^5 CFU of *S. mutans* and/or *Lactobacillus* per millilitre of saliva indicated a high risk for dental caries.[9]

The collected saliva was utilised to determine the buffering capacity (BC) of saliva. The saliva was pipetted on top of a test strip provided in the CRT kit and allowed to incubate for 5 min. During these 5 min, the strip changed colour, and the (BC) category of the saliva was recorded according to the manufacturer's instructions. A green colour was chronicled as a high (BC), a yellow colour indicated medium (BC), and a blue colour denoted a low (BC). A unanimous consensus among the three investigators was used to determine the scores and categories for the results.

Data were analysed utilising the SPSS[f] 21.0 statistical program. Descriptive analysis were performed using measures of central tendency followed by inferential statistics. A non-parametric randomised block analysis of variance (Friedman test) was used to compare observations repeated on the same subjects. The sign rank test was also applied to calculate intra-group differences. A p-value ≤ 0.05 was accepted as the significance level to control for the alpha level.

Results

The study was performed over 2 months at the College of Dentistry, Taibah University. A convenient sample of 21 participants was invited to participate. The female: male (F/M) was 8/13, and the median age was 30 years, ranging from 22 to 52 years, the mean age 32.6 ± 8.02.

The participants' tea consumption ranged from one cup/day (284 ml) to 11 cups/day with an average of 3.10 ± 2.75 cups/day. The sugar consumption per cup ranged from no spoons of sugar to 3 spoons per cup with a mean of 1.95 ± 0.95 spoons of sugar.

Table 1 shows the characteristics of the study sample regarding oral hygiene and tea drinking habits (Table 1).

The Caries Risk Test (CRT) Bacteria test results were considered to be low if the number of CFUs was less than 10^5 CFUs, or high if the number of CFUs was higher than 10^5 CFUs. Differences in *S. mutans* and *Lactobacillus* CFU density (CFUs/mL) before tea consumption, immediately after consumption, and 1 h after tea consumption, are summarised in Figures 1 and 2, respectively, and Table 2.

There were no statistically significant differences in *S. mutans* CFU density (CFUs/mL) between the various test periods (p > 0.05). Also, there was no statistically significant difference in CFU density (p > 0.05) before tea consumption and 1 h after tea consumption, or immediately after consumption and 1 h after tea consumption (P > 0.05).

Similarly, no statistically significant differences were observed in *Lactobacillus* CFU density (CFU/mL) between the various test periods. The differences before tea consumption and immediately after consumption were not statistically significant (P > 0.05), nor before tea consumption and 1 h after tea consumption (P > 0.05), nor immediately after consumption and 1 h after tea consumption (Table 2).

Figure 3 shows the lack of statistically significant difference in pH in the three test periods (p = 0.239) (NS).

**Table 1: Characteristics of the study participants.**

Variable	n	%
Tooth Brushing	21	100.0
Miswak	6	28.6
Flossing	3	14.3
Interdental sticks	5	23.8
Charcoal	2	9.5
Just water	8	38.1

Table 2: Distribution of *Streptococcus mutans* and *Lactobacillus* Colony Forming Units Before tea consumption, Immediately after consumption, and One hour after tea consumption.

Bacteria	Colony forming units (CFU)	Before tea consumption		Immediately after consumption		One hour after tea consumption	
		n	%	n	%	n	%
Streptococcus mutans	Less than 10^5	12	57.1	15	71.4	18	85.7
	More than 10^5	9	42.9	6	28.6	3	14.3
Lactobacillus	Less than 10^5	12	57.1	13	61.9	13	61.9
	More than 10^5	9	42.9	8	38.1	8	38.1
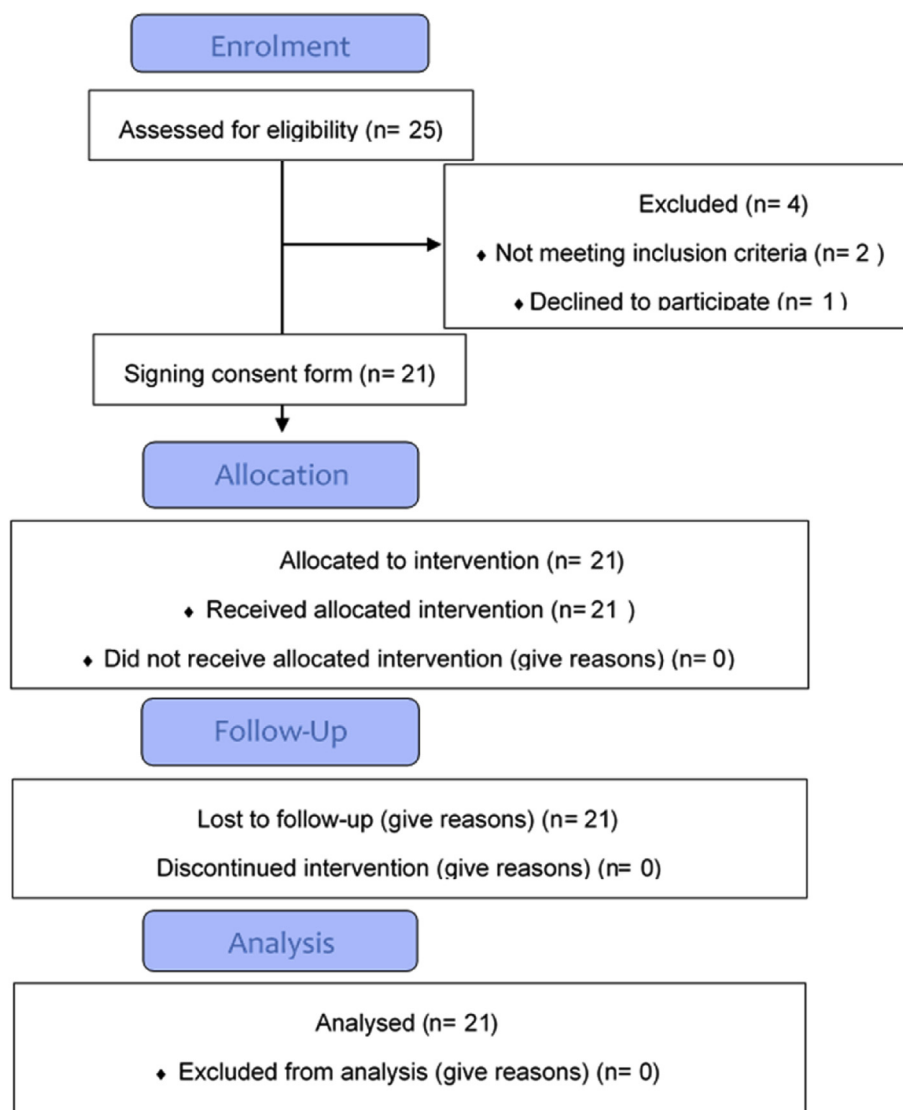
Figure 1: Flow Diagram: The influence of black tea on *Streptococcus mutans* and *Lactobacillus* levels in a population in Almadinah Almunawwarah, KSA.

Discussion

The present study was conducted to assess the antimicrobial activity of black tea on *S. mutans* and *Lactobacillus* in adults as a clinical indicator of the present caries condition, before-tea consumption, and at 1 and 2 h after tea consumption (see Figure 4).

Dental caries, an infectious multifactorial microbiologic disease of the teeth, occur due to the acid produced when sugars interact with bacteria, resulting in decalcification of the tooth structure. This condition is a major global oral health problem in developed and developing countries, affecting between 60 and 90% of schoolchildren and the majority of adults.[23] The primary aetiological agents
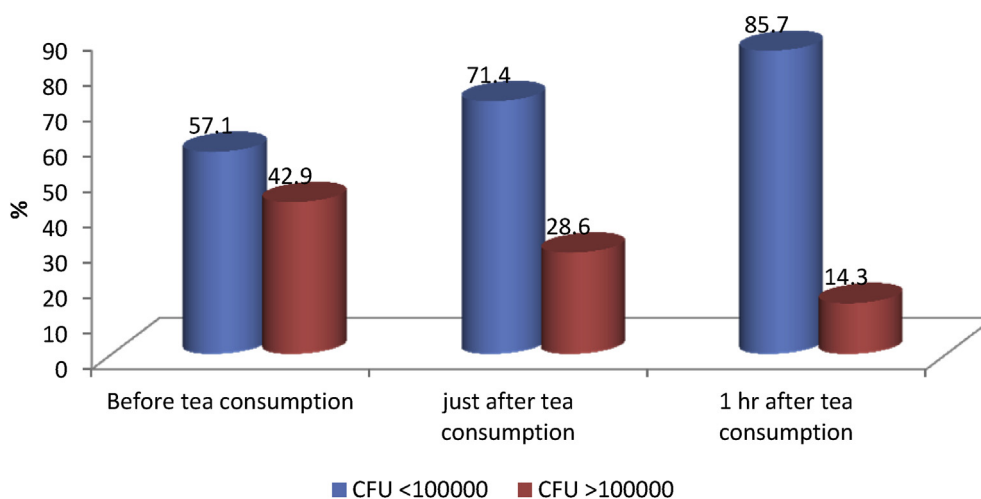
Colony Forming Units of S. mutans

Figure 2: **The distribution of *Streptococcus mutans* colonies (CFU density) Before, Immediately after, and 1 h after tea consumption.**[g] *Freidman Test − Streptococcus mutans: (P = 0.105 − NS). Related Sample Test Before tea consumption, Immediately after consumption and 1 h after tea consumption − Streptococcus mutans: Streptococcus mutans Before tea consumption and Immediately after consumption (P = 0.508 − NS). Streptococcus mutans Before tea consumption and 1 h after consumption (P = 0.453 − NS). Streptococcus mutans Immediately after consumption and 1 h after consumption (P = 0.070 − NS).*
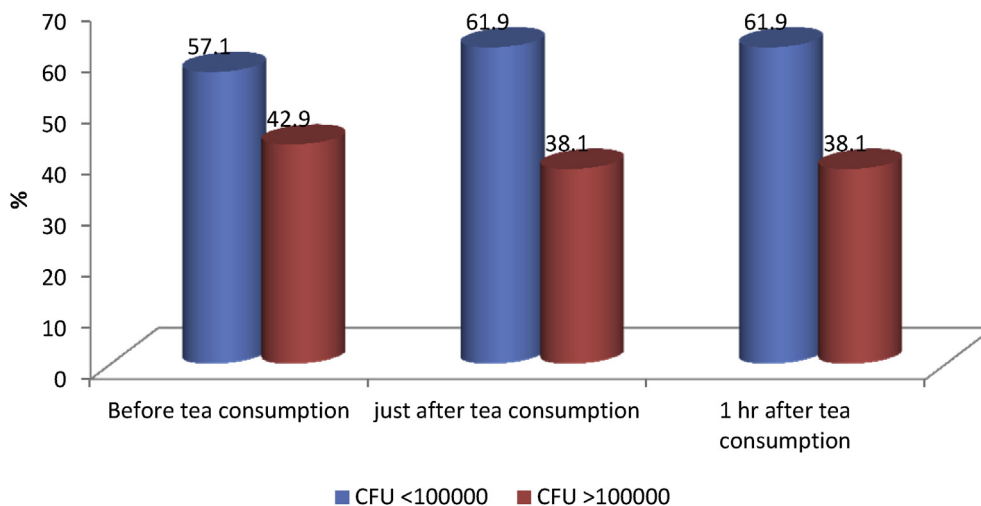
Colony Forming Units of Lactobacillus spp

Figure 3: **The distribution of *Lactobacillus* colonies (CFU density) Before, Immediately after, and 1 h after tea consumption**.[g] *Freidman Test − Lactobacillus: (P = 0.846 − NS). Related Sample Test Before tea consumption, Immediately after consumption and one hour after tea consumption − Lactobacillus: Lactobacillus Before tea consumption and Immediately after consumption (P = 1.000 − NS). Lactobacillus Before tea consumption and 1 h after consumption (P = 1.000 − NS). Lactobacillus Immediately after consumption and 1 h after consumption (P = 1.000 − NS).*

producing dental caries agents are restricted to several known strains of bacteria present in the dental biofilm (plaque) on tooth surfaces.[16,18,20,24,25]

Numerous microorganisms have been isolated from carious lesions. *S. mutans* and *Lactobacillus* spp are the central pathogenic species involved in the initiation and development of dental caries. AbdAllah et al. found that the salivary buffering capacity as well as *S. mutans* and *Lactobacillus* levels are significant risk factors for the development of dental caries. Furthermore, *S. mutans* and *Lactobacillus* promote caries due to the existence of surface-adsorbed salivary amylase, sucrose- and starch-producing

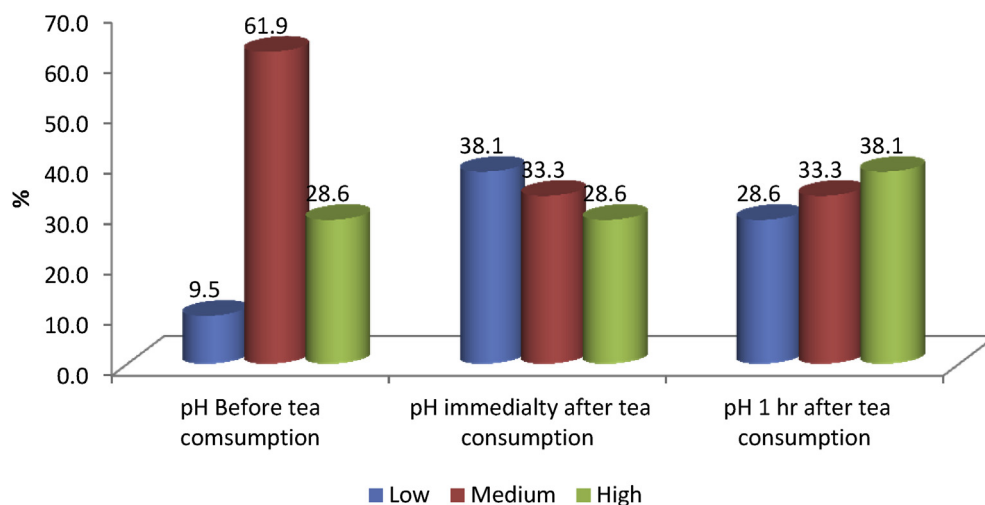# Distribution of Salivary pH Before, Immediately and 1 hr after tea consumption

Figure 4: Distribution of Salivary pH Before Immediately after, and 1 h after tea consumption. *Freidman Test − pH (P = 0.239 − NS). Related Sample Test Before tea consumption, Immediately after consumption and one hour after tea consumption − pH. pH Before tea consumption and Immediately after consumption (P = 0.146 − NS). pH Before tea consumption and 1 h after consumption (P = 0.549 − NS). pH Immediately after consumption and 1 h after consumption (P = 0.774 − NS).*

glucosyltransferases, and fructosyltransferase, which synthesises water-insoluble and soluble linked glucan from sucrose. Glucan adhesion consequently results in the formation of dental plaque. The presence of *S. mutans* and *Lactobacillus* in the dental plaque produces organic acids, resulting in enamel demineralisation.[13,25]

The existing mainstream commercial antiplaque products are antimicrobial compounds. However, many antibiotic and chemical bactericides now in use may interrupt the healthy bacterial flora in the oral cavity, initiating the overgrowth of antibiotic-resistant bacteria and additional opportunistic pathogens such as *Candida albicans*.[26] Several thorough studies have considered using natural substances derived from food as a possibility to avoid the occurrence of candidiasis.[27,28]

There has been increasing research attention focused on using natural plant extracts, principally those comprising of phenolic compounds, with both antimicrobial and antioxidant properties. This increased attention is perhaps a result of the new information regarding the practical effects in humans. Polyphenols are a group of substances present in all vegetative plant organs, flowers, and fruits. In the human diet, polyphenols are principally consumed via tea, coffee, cereals, and fruit. The health benefits of dietary polyphenols have been reported by nutritionists.[29]

The present study examined the association between the use of specific foods and the reduction of cariogenic oral bacteria.[3] Although the findings of this study did not find any statistically significant effect of tea consumption on cariogenic bacteria, tea extracts may become used in the future because such products are reasonably safe, their

taste is accepted worldwide, and these extracts could be used at an affordable cost to prepare specific anti-cariogenic preparations.

The findings of the current study showed that black tea has a tendency (though statistically insignificant) to reduce *S. mutans* counts, and to a lesser extent, on *Lactobacillus* counts. This finding have been echoed by previous studies, including that by Linke et al. which suggested that despite dietary sugar intake, black tea can reduce the initiation of dental caries.[30] A study by Touyz et al. also demonstrated an anti-cariogenic effect of a two-week regimen of black tea in 18-day-old mice.[31]

However, Subramaniam et al., suggests that the polymerisation changes of catechins significantly influence the inhibitory effect on glucosyltransferases in *S. mutans*. Moreover, they suggested that both aqueous and organic tea extracts had variable zones of inhibition. Oolong tea demonstrated the most significant inhibition zone, followed by green tea, and black tea had the smallest zone of inhibition.[9]

Black tea beverage demonstrates numerous degrees of inhibition of the growth of *S. mutans* and *Lactobacillus* bacteria.[32,33] Though reduction rates were not statistically significant, these results still may provide evidence for the presence of antimicrobial compounds in tea that are useful to control bacteria associated with dental caries.[32−34] These compounds can degrade the cell wall, disrupt the cytoplasmic membrane, damage membrane proteins, and interfere with membrane-integrated enzymes, which may

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[g] Related samples Friedman's two-way analysis of variance by ranks.

eventually lead to cell death.[35] In general, these results agree with results of previous *in vitro* microbial studies which demonstrated that tea had high caries-resistance properties which are thought to be due to their high contents of fluoride and polyphenolic catechin components.[34,36,37]

The bioactive components of tea have been demonstrated to significantly hinder the formation of *Streptococci* and *Lactobacilli*, and there is growing evidence that they can also disturb the adhesion of these bacteria to tooth enamel or they can operate as inhibitors of glucosyltransferase and amylase.[34,38] This finding is reflected by the study of Hamdi et al. which found that black tea prevents biofilm formation better than green tea.[39] However, this finding is in contrast to a study by Rasheed et al. that demonstrated the limited antibacterial activity of black tea. Rasheed et al. further mentioned that since black tea is a product of the fermentation of green tea leaves, its antibacterial activity in the form of polyphenols, catechins, gallic acid, and theaflavins is modified.[40,41]

Even though black and green teas both contain equivalent quantities of flavonoids, their chemical structures differ. Green tea comprises more catechins (simple flavonoids). Moreover, as a result of the fermentation process involved in the production of black tea, the simple flavonoids are converted into aflavins and arubigins. Furthermore, the simple flavonoid content depends upon the geographic location, soil, climate, and whether the tea is blended or decaffeinated.[42]

Conclusion

In conclusion, the current study demonstrated that it is easy to measure and determine subtle variations in the CFU density (CFU/mL) of *S. mutans* and *Lactobacillus*. However, the current study has demonstrated that black tea consumption has an insignificant antimicrobial effect against *S. mutans* and *Lactobacillus* bacteria.

Source of funding

Conflict of interest

The authors declare that they have no competing interests.

Ethical approval

The participants after receiving both verbal and written explanations of the study protocol and its aims signed a written informed consent written in both English and Arabic before the start of the study. Permission was obtained from Taibah University, College of Dentistry, Research Ethics Committee (TUCD-REC) with the approval number; TUCDREC/05122013/Mahrous.

Recommendations

Further studies need to be conducted using different concentrations of black tea *in vivo*, and additional studies can be performed with different types of tea on different bacteria.

Due to its preventive actions, tea extracts can be included in oral hygiene products such as toothpaste, dental floss, or mouthwashes.

Authors' contributions

RAM, Research idea, data entry, writing the first draft of the manuscript. HAB, Data collection, revising the final draft of the manuscript. MMS, Applying for ethical approval, participated in study design, data entry, reference preparation, revising the final draft of the manuscript. HTM, Study design, planning and supervising data collection, statistical analysis, revising the manuscript and prepare it for publication, submission for publication and responding to reviewer's comments. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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