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Effect of Black Tea on Some Cariogenic Bacteria

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Abstract: The aim of this study was to evaluate the antimicrobial effect of black tea against *streptococcus mutans* and *lactobacillus* species in adult Egyptian citizens. The unstimulated saliva samples were obtained from participants (pre, immediately post and after 1 hour of tea drinking) by spit in sterilized containers. The samples were added to transporting media and transmitted to bacteriological laboratory for culturing and counting. Results showed that the black tea beverage had a highly significant effect on reducing the cariogenic bacterial counts. This reduction reached to 60 and 99.9% of *streptococcus mutans* and 91 and 98% of *lactobacillus* in the immediately post and after 1 h of tea drinking samples, respectively. Also, the moderate consumption of tea (3-4 cups/day) exhibited extremely low values of *lactobacillus* ($2.4x10^4$ - $4.7x10^3$) and DMF score (5.6). It could be concluded that, black tea exhibited strong antimicrobial effect against *streptococcus mutans* and *lactobacillus* bacteria. Consequently it is recommended as an effective natural beverage to combat dental caries.

Key words: Black tea · Streptococcus · Lactobacillus · Dental caries · DMF score

INTRODUCTION

Dental caries is one of the most common diseases in humans. It is an infectious microbiologic disease of the teeth that results in localized dissolution and destruction of the calcified tissue. Also, it is a multifactorial disease, which is caused by host, agent, time and environmental factors. A wide group of microorganisms are identified from carious lesions of which *streptococcus mutans* and *lactobacillus* are the main pathogenic species involved in the initiation and development of dental caries. Many studies suggested that *mutans streptococci, Lactobacillus* and salivary buffering capacity are important risk factors for dental caries [1-4].

Streptococcus mutans is considered as the major etiological pathogen of dental caries. It was classified into 3 serotypes C, E and F due to the different chemical compositions of the serotype-specific polysaccharides. It possesses a variety of mechanisms to colonize tooth surfaces [5-7]. These bacteria in presence of surface-adsorbed salivary α-amylase, sucrose and starch can produce bacterial enzymes such as glucosyltransferases and fructosyltransferase that synthesize water-insoluble and -soluble α-linked glucans from sucrose. They adhere on the tooth surface with other oral bacteria. Consequently, the adhesion of glucan brings about the formation of dental plaque. Furthermore, these bacteria in dental plaque produce organic acids which cause the enamel demineralization [8,9].

Lactobacillus genus includes more than 80 species so their taxonomy is not easy because a lot of different Gram+ bacilli are grouped together under this name [10,11]. Although it is not present in the oral cavity at birth, it transmitted to the oral cavity during the first years of a child's life from surrounding individuals [12]. A strong correlation has been established between the saliva Lactobacillus count and dental caries [13]. *Lactobacilli* are considered secondary invaders, rather than initiators, in the caries process [14] so the presence of *lactobacilli* is dependent on the cavity size whereas

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they are present more in medium and large cavities. Also, it present in root caries and in deep dentinal caries associated with pulpitis [15,16]. Pits and fissures or partially erupted third molars provide a retentive environment favorable to the growth of these microorganisms [14,17].

Many compounds from natural products have been extensively surveyed to control the dental caries; only a very restricted number of these are available for medical applications because of effectiveness, stability, odor, taste and economic feasibility. Tannic acid, found in tea phenols, is an important inhibitor of bacterial growth and glucosiltransferase activity [18,19].

Tea is consumed all over the world, whereas it has been used for medicinal purposes for thousands of years, possibly as long ago as 2700 BC. Tea is the world's most widely consumed beverage; more than two billion cups are drunk daily. Of the tea produced worldwide, 78% is black tea, 20% is green tea and 2% is Oolong tea [20].

Tea has been found to exhibit various bio-regulatory activities such as anti-carcinogenetic [21-25], anti-metastatic [26,27], anti-oxidative [28,29], anti-hypertensive [30], anti-hypercholesterolemic [31], anti-dental caries [32] anti-bacterial [33] and to contribute to intestinal flora amelioration activity [34]. Also, tea consumption is linked to lower incidences of various pathological conditions, including cardiovascular disease [35], strokes [36], obesity [37], diabetes [38], inflammatory conditions [39] and aging process [40].Moreover, tea has been shown to afford significant protection against Parkinson's disease, Alzheimer's disease and ischemic damage [41].

The chemical composition of tea is complex: proteins (10-15%); amino acids (1-4%) such as theanine or 5-*N*-ethylglutamine, glutamic acid, tryptophan, glycine, serine, aspartic acid, tyrosine, valine, leucine, threonine, arginine and lysine; carbohydrates (5-7%) such as cellulose, pectins, glucose, fructose and sucrose; minerals and trace elements (5%) such as calcium, magnesium, chromium, manganese, iron, copper, zinc, molybdenum, selenium, sodium, phosphorus, cobalt, strontium, nickel, potassium, fluorine and aluminum; trace amounts of lipids (linoleic and α-linolenic acids); vitamins (B, C, E); pigments (chlorophyll and carotenoids) and volatile compounds (aldehydes, alcohols, esters, lactones and hydrocarbons) [29,42]. In addition, there are polyphenolic compounds including catechins (epicatechin, epicatechin-3-gallate, epigallocatechin and epigallocatechingallate) [42,43], flavonoids (quercetin, kaempferol, myricetin and their glycosides), phenolic acids (such as tannic acid), caffeine and dietary fluoride [43,44]; these compounds may account for up to 30% of the dry weight.

There have been many studies of the antimicrobial effects and antioxidant activities of tea on animal models. However, there is paucity of information regarding the inhibitory effects of tea constituents on cariogenic bacteria *in vivo*. Therefore, the aim of this study was to evaluate the antimicrobial activity of black tea on *streptococcus mutans* and *lactobacillus sp.* in some adult Egyptian citizens.

MATERIALS AND METHODS

Materials: Fine black tea (Lipton tea, imported and packed in Egypt by Unilever Mashreq Co.) and sugar were purchased from a local supermarket, Cairo, Egypt. Mitis salivarius agar medium (made by Diffico Co. USA) was used as selective medium for *streptococcus mutans* purchased from Trading Dynamic Co., Giza, Egypt. While, tomato agar medium was used as selective medium for *lactobacillus* prepared by the bacteriological laboratory, Faculty of Medicine, Al-Azhar University. Sterile plastic syringes were imported from Shandong Zibo Shanchuan med. instrument Co., Ltd. China.

Methods: Preparation of Tea Beverage: The tea beverage was prepared according to the traditional Egyptian method with 2 g of black tea, 12 g of sugar (3 spoons) and about 180 ml of boiled water.

Individuals Selection: Eighty seven adult citizens (32 female and 55 male aged 13-71 years) participated in this study and selected according to the following criteria:

Medical History: Individual had no remarkable medical history or history of drug administration as antibiotics administration or any other drugs that could affect his immunity or the oral microorganism's counts in the last 3 weeks. Individuals who suffered from systemic ailments such as diabetes, cardiopathy, renal alterations and any immunocompromised disease were excluded.

Dental History: Individuals were had permanent dentations only and free of oral inflammation or any oral septic foci that could affect the numbers of oral microorganisms.

Data Collection: A personal data including name, age, sex…ect was taken. Clinical DMF (Decayed, Missed and Filled tooth) score was recorded according to criteria of the World Health Organization for permanent dentition [45]. The participants were asked about the following:

- Habit of tea drinking (drinker or none drinker).
- Period of tea drinking. The drinker group was classified into four subgroups: G1: 1-5y, G2: 6-10y, G3: 11-20y and G4: more than 20y.
- Number of tea cups consumed/day.
- Number of sugar spoons/cup.
- Temperature of tea beverage that the individual like to drink.

The relation of the previous factors to oral normal bacterial counts present in the initial saliva sample and DMF score was studied and tabulated.

Samples Collection: Three samples (pre, immediately post and after 1h of tea drinking) of fresh unstimulated saliva (first thing in the morning or at least 2 hours after meal) were obtained from each participant by spiting in a sterilized container [46]. Then with a sterile plastic disposable syringe, 1ml of the collected saliva sample was added to a tube containing 9ml thioglycolate broth medium as a transfer medium. Afterwards, the samples were transmitted immediately to the bacteriological laboratory, Faculty of Medicine, Al-Azhar University for culturing and counting.

Bacteriological Investigation: The thyoglycolate broth containing saliva samples were diluted 1:10, 1:100 1:1000 and 1:10000 in sterile saline. Then, l ml of each dilution of saliva specimen was homogeneously spread on the surface of the selective media. The plates were incubated aerobically and anaerobically at 37°C for 48-96 h for *streptococcus mutans* and at 37°C for 96 h in an anaerobic atmosphere for *lactobacillus* [47]. Then the colonies were counted and calculated.

Statistical Analysis: The collected data were statistically analyzed using the Student's t-test and one-way analysis of variance (ANOVA) followed by the Fisher's exact test. Values of $P<0.05$ were considered significant [48].

RESULTS

The influence of tea beverage on the bacterial counts of saliva samples is presented in table 1. A great decrease ($P<0.05$) in bacterial counts was detected in all samples either immediately post or after 1 h of tea drinking. The reduction rates reached to 60 and 99.9% of *streptococcus mutans* and 91 and 98% for *lactobacillus* in the samples post immediately and after 1 h, respectively as shown in fig. 1.

Table 1: The effect of black tea on the counts of *s. mutans* and *lactobacillus*

	SM			LB		
Count	Pre	Post	After 1 h.	Pre	Post	After 1 h.
Min.	$3x10^2$	$2x10^2$	$1x10^2$	$1x10^3$	$3x10^2$	$1x10^2$
Max.	$8x10^6$	$3x10^6$	$2x10^5$	$4x10^6$	$4x10^5$	$2x10^4$
Mean	$1x10^6$	$4x10^5$	$1x10^3$	$1x10^5$	$9x10^3$	$2x10^3$
SD	$1x10^6$	$7x10^5$	$3x10^4$	$5x10^5$	$4x10^4$	$3x10^3$
P* 1:2	0.000			0.004		
1:3	0.000			0.002		
2:3	0.004			0.878		

*1: pre-drinking sample, 2:post-drinking sample and 3: after 1h saliva sample

Table 2: The effects of tea consumption and temperature on the bacterial counts and DMF scores

	SM				LB				DMF			
Group	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD
*G 1	$3x10^2$	$8x10^6$	$1.1x10^6$	$1.5x10^6$	$1x10^3$	$4x10^6$	$6.1x10^4$	$7.3x10^4$	0	22	6.4	4.9
G 2	$2.4x10^5$	$4x10^6$	$2.1x10^6$	$1.4x10^6$	$4x10^3$	$2x10^5$	$1.5x10^5$	$5.4x10^5$	2	21	11.1	5.3
P			0.794				0.238				0.789	
Temperature												
Hot	$7x10^2$	$4x10^6$	$1x10^6$	$1.4x10^6$	$1x10^3$	$3x10^5$	$5x10^4$	$9.5x10^4$	0	22	5.5	4.7
Mod.	$3x10^2$	$4x10^6$	$1.1x10^6$	$1.6x10^6$	$1x10^3$	$4x10^6$	$2x10^5$	$9x10^4$	0	15	7.0	4.9
P			0.991				0.022				0.261	

*G 1: group drink tea and G 2: group not drink tea

Table 3: The effects of tea cups, sugar spoons and drinking years on both the bacterial counts and DMF scores

	MS				LB				DMF			
	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD
*Cups												
1	$3x10^2$	$4x10^6$	$6x10^5$	$1x10^6$	$2x10^3$	$2x10^6$	$1.4x10^5$	$4.4x10^5$	0	22	9.6^a	5.2
2	$2x10^3$	$4x10^6$	$8.9x10^5$	$1.3x10^6$	$1x10^3$	$9x10^5$	$1.1x10^5$	$2.4x10^5$	0	15	7.6^a	5.2
3	$7x10^2$	$4x10^6$	$1.2x10^5$	$1.6x10^6$	$1x10^3$	$4x10^6$	$2.4x10^5$	$8.1x10^5$	0	14	5.6^a	4.5
4≥	$2x10^3$	$8x10^6$	$1.8x10^6$	$2.2x10^6$	$2x10^3$	$2x10^5$	$4.7x10^4$	$9.2x10^4$	0	15	5.6^a	4.7
*Spoons												
1	$2x10^3$	$4x10^6$	$1.5x10^6$	$1.5x10^6$	$1x10^3$	$1x10^4$	$5.3x10^3$	$4x10^3$	0	13	5.0^a	5.1
2	$7x10^2$	$4x10^6$	$6.2x10^5$	$1.2x10^6$	$2x10^3$	$3x10^5$	$7.9x10^4$	$1x10^5$	0	15	7.4^a	7.4
3	$3x10^2$	$8x10^6$	$1.2x10^6$	$1.7x10^6$	$1x10^3$	$4x10^2$	$4x10^5$	$7.1x10^5$	0	22	6.1^a	4.8
4≥	$2x10^3$	$4x10^6$	$1.1x10^6$	$1.4x10^6$	$2x10^3$	$3x10^5$	$1x10^4$	$1x10^4$	0	11	5.2^a	3.4
Years												
G1	$3x10^2$	$4x10^6$	$9.5x10^{5a}$	$1.3x10^6$	$1x10^3$	$4x10^6$	$3.3x10^{5a}$	$9.1x10^5$	0	22	10.3^a	5.0
G2	$3x10^3$	$4x10^6$	$1.2x10^{6a}$	$1.5x10^6$	$1x10^3$	$3x10^5$	$5.4x10^{4a}$	$1x10^5$	0	10	3.5^b	3.2
G3	$7x10^2$	$3x10^6$	$9.1x10^{5a}$	$1.2x10^6$	$1x10^3$	$3x10^5$	$4.8x10^{4a}$	$9.7x10^4$	0	12	2.8^b	4.1
G4	$2x10^3$	$8x10^6$	$1.1x10^{6a}$	$2x10^6$	$1x10^3$	$9x10^5$	$9.2x10^{4a}$	$2x10^5$	0	11	4.8^b	4.2

* No differences detected between groups**G 1: 1-5y, G 2: 6-10y, G 3: 11-20y and G 4: more than 20y

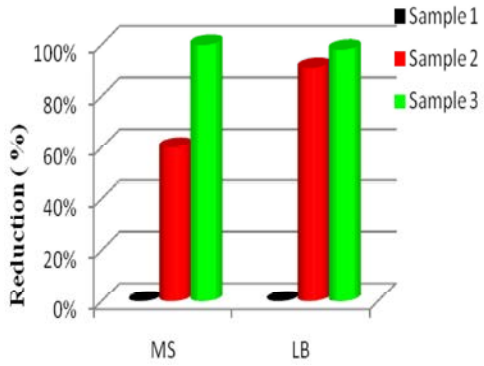

Fig. 1: Reduction % in bacterial counts of saliva samples

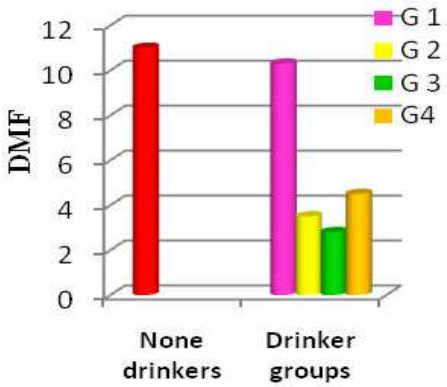

Fig. 2: Relation of tea consumption rates to DMF scores

The bacterial counts and mean DMF scores were insignificantly lower in tea drinker group as comparing with the none drinker group as shown in table 2 and fig. 2. Also, the same table indicated that drinking hot tea could slightly decrease *s.mutans* and DMF scores. Moreover, the mean *lactobacillus* count was significantly lower ($P<0.05$) in group drink hot tea than group drink tea of moderate temperature.

In regard to number of tea cups and sugar spoons, no differences were detected among the initial bacterial counts and DMF scores of the classified groups as shown in table 3. Also, no significant differences were observed among CFU/mL values of *s.mutans* and *lactobacillus* with the increase of tea consumption years. Concerning the relation between DMF scores and drinking years, it is clear that there was a significant difference ($P<0.05$) found between first group and the other three groups as demonstrated in table 3.

DISCUSSION

In the last few years, an increased attention has been focused on the natural plant extracts, especially those containing phenolic compounds with antimicrobial and antioxidant properties. Tea is one of the important dietary sources of these compounds [42-44].

Black tea beverage showed various degrees of inhibition against the growth of *s. mutans* and *lactobacillus* bacteria. Whereas, reduction rates were 60 and 99.9% of SM, while 91 and 98% of LB in the samples immediately post and after 1 h, respectively. These results provide evidence for the presence of antimicrobial phenolic compounds in tea which are useful in the control of common oral infections, especially dental caries

bacteria [49]. These compounds can degrade the cell wall, disrupt the cytoplasmic membrane, damage membrane proteins and interfere with membrane-integrated enzymes, which may eventually lead to cell death [50]. Generally, these results are in agreement with Hashimoto *et al.* [29], Chung *et al.* [44] and peter *et al.* [49], they demonstrated *in vitro* microbial studies that tea had high caries resistances properties. This due to their high contents of fluoride and polyphenolic catechin components. Previous studies [18-21] indicated that the tannic acid is an important inhibitor of bacterial growth and glucosiltransferase activity. This acid is also able to form stable complexes with proteins rich in proline that are present in saliva and are directly involved in the adherence of oral bacteria to the acquired pellicle.

In this study, The bacterial counts and mean DMF scores were lower in subjects drink tea than none drinking group. These results were similar to previous clinical studies [21,22,51], which indicated that significantly lower DMF and plaque scores in American individuals who drank tea (1-3 cups/day) than none drank. There are convincing evidences that the bioactive components of tea are able to inhibit proliferation of the streptococci and Lactobacilli agents, interfere with the process of adhesion to tooth enamel or act as inhibitors of glucosyltransferase and amylase [18,19,49].

Regarding tea temperature, the present results showed that hot tea reduce of the bacterial counts and DMF scores by different rates when compared with moderate tea temperature. This reduction in bacterial counts may be referred to rise of the oral tissues temperature that can affect the growth and multiplication of oral microorganisms. This suggestion was in agreement with De Jong *et al.* [52] who mentioned that drinking hot beverages could substantially increase the intra-esophageal temperature by 6-12 °C, depending on the sip size.

The current study found a negligible effect of numbers of tea cups and sugar spoons on the initial bacterial counts and DMF scores. The moderate consumption of tea (3-4 cups/day) exhibited extremely low values of *lactobacillus* ($2.4x10^4$ - $4.7x10^3$) and DMF score (5.6) when compared with groups given 1-2 cups/day. These results are in agreement with Ramsey *et al.* [53] who reported that there significant inverse correlation was found between amounts of tea drunk daily and DMF score. They attributed this effect to increase the time of fluoride intake. Moreover, a significant inverse relation was observed between DMF scores and years number of tea consumption. Since, the mean DMF scores gradually reduced (from 10.3 to 2.8) with the increase of tea consumption years.

It could be concluded that, black tea exhibited strong antimicrobial effect against *streptococcus mutans* and *lactobacillus* bacteria. Also, the moderate consumption of tea (3-4 cups/day) exhibited extremely low values of *lactobacillus*. In addition it showed significantly lower DMF scores in tea drinker individuals. So it is recommended as effective natural measure against dental caries, consequently improve the oral health.

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