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Antimicrobial Effect of Tea and Tea with Milk Beverages on Oral *Streptococcus mutans* and *Lactobacilli*

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Abstract: The aim of this study was to compare the antimicrobial effects of black tea to that of tea with milk (TM) against oral *Streptococcus mutans* and *Lactobacillus* sp. in Egyptian children. Three saliva samples were obtained from children (pre, immediately post and after 1 hour of beverage drinking) by spiting in sterilized containers. The samples were added to transporting media and transmitted to bacterial lab for culturing and counting. The results showed that tea and TM beverages had a highly significant bacterial counts reduction against these cariogenic bacteria by different rates (43.6% - 83.3%). Conclusions; tea and TM exhibited a magnificent antimicrobial effect against *S. mutans* and *Lactobacillus* bacteria. Consequently, they are recommended as effective natural anti-cariogenic beverages.

Key words: Black Tea · Milk · Streptococcus · Lactobacillus · Dental Caries · Dmf · DMF

INTRODUCTION

Dental caries and pulpal diseases are the most common oral bacterial diseases. It is an infectious transmissible disease where a cariogenic biofilm results in localized demineralization and destruction of dental hard tissue. Also, it is a multifactorial disease, which is affected by host, agent, time and environmental factors. Many studies have confirmed *streptococcus mutans* (SM) and *lactobacillus* (LB) as the sole causative microbiological agents and their presences in the dental structure cariogenic biofilm are indicator of dental caries [1-3].

Streptococcus mutans is considered as the major initiator pathogen of dental caries and classified into 3 serotypes C, E and F according to the different chemical composition of the serotype-specific polysaccharides. It possesses a variety of mechanisms to colonize tooth surfaces [4, 5]. 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. Glucans 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 [6].

Lactobacilli are Gram-positive bacteria constituting part of the normal oro-gastrointestinal flora. They include more than 80 species so their taxonomy is not easy [7, 8]. *Lactobacilli* are not present in the oral cavity of the child at birth; they are transmitted to the oral cavity during the first years of a child's life from surrounding individuals [9]. A strong correlation has been established between the saliva Lactobacillus count and dental caries [10]. *Lactobacilli* in the caries process are considered secondary invaders, rather than initiators [11], so the presence of *lactobacilli* is dependent on the cavity size where they are present more in medium and large cavities. Pits and fissures provide a retentive environment favorable to the growth of these microorganisms [12].

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Recently, the interest in therapeutic plants has increased dramatically [13] as 80% of the world's people rely on complementary and alternative medicine for their health care needs [14]. Tea polyphenols are important inhibitors of bacterial growth and glucosyltransferase activity [15-17].

Tea is the most popular non-alcoholic and healthy beverage across the world. Of tea consumed in the world, 78% is black tea [18]. Tea exhibited various bio-regulatory activities such as antibacterial [15, 17], antifungal [19], anti-inflammatory [20], antiviral [21], anti-cariogenic [22, 23], antioxidant [23, 24], anti-carcinogenic [25, 26] and anti-hypertensive [27]. Also, tea reduce incidence of various pathological conditions, including cardiovascular diseases [28, 29], brain and nervous disorders, various infections (such as throat infections, dental abscesses and mouth ulcers) [20], high blood pressure [29], lipid parameters [30], HDL cholesterol [31], obesity [24], diabetes [32] and aging process [33]. Moreover, it affords significant protection against Parkinson's disease, Alzheimer's disease and ischemic damage [34].

Tea contains bioactive compounds such as flavonoids that are classified into flavonols, flavones, flavan-3-ols, flavanones, anthocyanidins and isoflavonoids [16, 35]. Flavan-3-ols includes: catechin (about 70% from polyphenols), epigallocatechin, epicatechin, epicatechin 3-gallate, epigallocatechin 3-gallate, theaflavin, thearubigins, theaflavin-3, 3-digallate, theaflavin-3-gallate, theaflavin-3'-gallate, gallocatechin and catechin 3-gallate [17, 36]. Also, it contains phenolic acids (such as tannic acid), caffeine and dietary fluoride [37]; these compounds may account for up to 30% of the dry weight.

Tea with milk (TM) is a common beverage. Although controversy studies about adverse reactions of milk proteins with tea showed that proteins and Fe present in milk react with tea polyphenols and tannic acid forming insoluble complexes (polyphenol-protein and ferric taninate) that reduce the intestinal absorption of polyphenols and Fe [38, 39]. In addition, milk caseins prevent vascular protective effects of tea catechins [40]. On the other hand, studies demonstrated that the absorption and bioavailability of tea flavonols are not affected by addition of milk consequently, their antioxidant properties [41]. Moreover, other studies reported that the moderate intake (less than 10g/kg diet) of tannic acid is relatively safe, while the high intake reduces the hemoglobin concentration [42, 43].

Recently, there are studies showed that daily consumption of milk and dairy products may reverse soft and leathery caries and decrease the salivary levels of *mutans streptococci* in adults [44]. In addition, there are many studies about the antimicrobial effect of tea [17, 22, 45] but, there is an information paucity on regarding the inhibitory effects of tea with milk beverage constituents on cariogenic bacteria *in vivo*. Hence, the purpose of the study was to compare antimicrobial effects of black tea and tea with milk (TM) beverages on oral *Streptococcus mutans* and *Lactobacillus* sp. in Egyptian children.

MATERIALS AND METHODS

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

Methods

Preparation of Beverages: The beverages of tea and tea with milk (TM) were prepared according to the traditional Egyptian methods. Tea beverage prepared by adding 2 g of black tea to about 180 ml of boiled water. While, TM beverage was prepared by mixing 90 ml tea beverage with 90 ml boiled milk. Both beverages were sweetened with 12 g (3 small spoons) of sugar.

Individuals Selection: Ninety children of both sexes (28 female and 62 male aged 4 - 12 years) participated in this study. The children were equally divided into two groups, the first group drunk tea beverage, while the other group drunk TM beverage. The children selected according to the following criteria:

***Medical History*:** The child who had no remarkable medical history or history of drug administration as antibiotics or any other drugs that could affect his immunity or the oral microorganism's counts in the last 3 weeks was selected. Children who suffered from

systemic ailments such as diabetes, cardiopathy, renal alterations and any immunocompromised disease were excluded.

***Dental History*:** Children free of oral inflammation or any oral septic foci that could affect the numbers of oral microorganisms were included in the study.

Data Collection: A personal data including name, age, sex…ect was taken. Decayed, Missed and Filled teeth (DMF for permanent dentition) index and decayed, missed and filled (dmf for primary dentition) index scores were calculated according to WHO criteria of caries indices [46]. The children or their parents were asked about the following:

- Habit of beverage drinking (drinker or non drinker).
- Drinking period: The drinker group were classified according to drinking period into 2 subgroups: G1: 1-2 y and G2: more than 2 y.
- Number of beverage cups consumed/day.
- Number of sugar spoons/cup.
- Temperature of beverage that the child likes to drink.

The relations of the previous factors to normal oral bacterial counts present in the initial saliva sample and DMF or dmf scores were studied and tabulated.

Samples Collection: Three saliva samples were obtained from each child by spiting in a sterilized container. The first sample (pre.) was taken first thing in the morning or at least 2 hours after meal (fresh unstimulated saliva). The second sample obtained (post.) immediately post drinking, while the third sample obtained after 1 h of beverage drinking [47]. Then with a sterile plastic disposable syringe, 1ml of the collected saliva sample was added to a tube containing 9 ml 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: Thioglycolate broth containing saliva samples were serially diluted 1:10, 1:100 1:1000 and 1:10000 in sterile saline. Then, 1 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 37ºC for 48-96 h for *S. mutans* and for 96 h in an anaerobic atmosphere for *lactobacillus* [48]. Then the colonies were counted and calculated.

Statistical Analysis: The data were statistically analyzed by using SPSS (Version 16.0 software Inc., Chicago, USA) of completely randomized design as described by Gomez and Gomez [49]. Treatment means were compared using the least significant differences (LSD) at 0.05 levels of probability and standard error.

RESULTS

Both beverages showed a significant reduction of bacterial count ($P< 0.05$) in all samples either immediately post or after 1 h of beverage drinking as shown in Table 1. However, tea beverage demonstrated a higher anti-cariogenic effect where it reported 51.1 and 83.3% reduction of *S. mutans* and 57.7 and 86.7% of *lactobacillus*, respectively. On the other hand, TM beverage reported 43.6 and 79.1% of *S. mutans* and 51.6 and 77.4% of *lactobacillus,* respectively as shown in Fig. 1.

Table 1: Effect of tea and TM beverages on the counts of *S. mutans* and *Lactobacillus* in saliva samples.

		SM			LB		
Beverage		Pre	Post	After 1 h.	Pre	Post	After 1 h.
Tea	Min.	0	0	0	0	0	0
	Max.	$3.5x10^5$	$2x10^6$	$8x10^5$	$5x10^5$	$2x10^5$	$1x10^5$
	Mean	$9x10^{5a}$	$4.4x10^{4b}$	$1.5x10^{4c}$	$9x10^{4a}$	$3.8x10^{3b}$	$1.2x10^{3b}$
	SD	$8.5x10^4$	$4.3x10^4$	$1.9x10^4$	$9.6x10^3$	$5.1x10^3$	$2.1x10^3$
TM	Min	0	0	0	0	0	0
	Max.	$1.3x10^6$	$3.1x10^5$	$1.2x10^5$	$4x10^5$	$3x10^5$	$2x10^5$
	Mean	$1.1x10^{5a}$	$6.2x10^{4ab}$	$2.3x10^{4b}$	$6.2x10^{3a}$	$3x10^{4b}$	$1.4x10^{3b}$
	SD	$2x10^6$	$6.6x10^4$	$2.9x10^4$	$7.6x10^3$	$4.8x10^3$	$3.2x10^3$

[a],[b]and[c] means in the same raw with different superscripts are different significantly ($p<0.05$).

Table 2: Effects of beverage consumption and temperature on both bacterial counts and dmf+DMF scores.

		Tea					Tea with milk (TM)				
		Consumption		Temperature			Consumption		Temperature		
		*G 1	G 2	Hot	Mod.	Cold	*G 1	G 2	Hot	Mod.	Cold
SM	Min.	$4x10^4$	0	$4x10^4$	0	0	0	0	0	0	0
	Max.	$3.5x10^5$	$3x10^6$	$3.5x10^5$	$3x10^6$	$3x10^6$	$3x10^6$	$1.3x10^6$	$1.5x10^5$	$3x10^6$	$7.2x10^5$
	Mean	$8.2x10^{4a}$	$9.4x10^{4a}$	$9.8x10^{4a}$	$7.9x10^{4a}$	$1x10^{6a}$	$6.4x10^{4a}$	$1.2x10^{5a}$	$6.2x10^{4a}$	$9.5x10^{4a}$	$1.2x10^{5a}$
	SD	$9.3x10^4$	$8.3x10^4$	$8.7x10^4$	$8.4x10^4$	$9.1x10^4$	$8.2x10^4$	$2.3x10^5$	$4.6x10^4$	$9.8x10^4$	$1.9x10^5$
LB	Min.	0	0	$1x10^4$	0	0	0	0	0	0	0
	Max.	$2.5x10^4$	$5x10^5$	$3x10^5$	$1.8x10^4$	$5x10^5$	$1.4x10^4$	$4x10^5$	$4x10^5$	$1.4x10^4$	$1.8x10^4$
	Mean	$7.2x10^{3a}$	$9.9x10^{3a}$	$1.2x10^{4a}$	$6x10^{4a}$	$1.1x10^{4a}$	$4.7x10^{3a}$	$6.8x10^{3a}$	$4.8x10^{3a}$	$6.6x10^{3a}$	$7x10^{4a}$
	SD	$6.9x10^3$	$1.1x10^4$	$7.6x10^3$	$6.6x10^3$	$1.4x10^4$	$4x10^4$	$8.6x10^3$	$1.1x10^4$	$4.6x10^3$	$5x10^4$
DMF	Min.	0	0	0	0	0	2	0	0	0	0
	Max.	13	14	10	14	11	12	11	10	11	12
	Mean	3.4^b	5.6^a	3.4^a	3.9^a	4.6^a	3.6^b	5.9^a	3.4^a	4.3^a	5.3^a
	SD	3.3	3.7	3.4	3.4	3.8	3.3	3.9	3.6	3.2	3.5

*G 1: group drunk beverage and G 2: group did not drink ^{a,b}and^c means in the same raw with different superscripts are different significantly ($p<0.05$)

Table 3: Effect of beverage drinking years on the bacterial counts and total dmf+DMF scores.

		SM				LB				dmf+DMF			
		Min.	Max.	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD
Tea	*G1	0	$3x10^6$	$1.2x10^{5a}$	$1x10^6$	0	$1.5x10^4$	$6.9x10^{3a}$	$5.7x10^3$	0	10	4.4^a	3.4
	G2	0	$2x10^6$	$7.9x10^{4a}$	$6.4x10^4$	0	$3x10^5$	$9.4x10^{3a}$	$8.7x10^3$	0	14	2.9^b	3.8
TM	G1	0	$4.1x10^5$	$8.7x10^{4a}$	$9.1x10^4$	0	$4x10^5$	$8.5x10^{3a}$	$9.7x10^3$	0	11	3.3^a	3.0
	G2	0	$3.3x10^5$	$9.7x10^{4a}$	$9.5x10^4$	0	$1.3x10^4$	$3.7x10^{4a}$	$5.1x10^3$	0	5	1.7^b	1.8

*G 1: 1-2 drinking years and G 2: 3 y or more. ^{a,b}and^c means in the same column with different superscripts are different significantly ($p<0.05$)

Table 4: Effects of beverage cups and sugar spoons on the bacterial counts and dmf+DMF scores

		Tea				Tea with milk (TM)			
		*Cup		**Spoon		*Cup		**Spoon	
Groups		1	2	1	2	1	2	1	2
SM	Min.	0	$2x10^5$	0	0	0	$2.5x10^3$	0	0
	Max.	$3x10^6$	$3x10^6$	$3x10^6$	$3x10^6$	$1.3x10^6$	$4.1x10^5$	$3.3x10^5$	$1.3x10^6$
	Mean	$9.9x10^{4a}$	$8.7x10^{4a}$	$9.9x10^{4a}$	$9.2x10^{4a}$	$1.4x10^{5a}$	$1x10^{6a}$	$6.3x10^{4a}$	$2.1x10^{5a}$
	SD	$8.7x10^4$	$8x10^5$	$9.9x10^4$	$7.7x10^4$	$2.9x10^5$	$1.2x10^5$	$8.2x10^4$	$3.4x10^5$
LB	Min.	0	0	$5x10^4$	0	0	0	0	0
	Max.	$5x10^5$	$2x10^5$	$5x10^5$	$3x10^5$	$2.5x10^4$	$4x10^5$	$4x10^5$	$1.7x10^4$
	Mean	$1.1x10^{4a}$	$7.8x10^{3a}$	$1.4x10^{4a}$	$8x10^{4a}$	$6.7x10^{3a}$	$7x10^{4a}$	$8.4x10^{3a}$	$5.3x10^{3a}$
	SD	$1.3x10^4$	$6.7x10^3$	$1.3x10^4$	$8.8x10^3$	$7x10^4$	$1.1x10^4$	$1.1x10^4$	$4.9x10^3$
DMF	Min.	0	0	0	0	0	0	0	0
	Max.	10	14	6	14	9	11	10	11
	Mean	3.5^a	3.4^a	2.6^a	3.9^a	3.9^a	3.4^a	2.3^a	5.0^a
	SD	4.3	3.0	2.3	3.0	4.4	2.6	2.8	3.4

*G1: 1cup/day G2: 2 or more /day. **G1:1-2 spoon /cup G2: 3or more /cup

a, b and c means in the same row with different superscripts are significantly different ($p<0.05$).

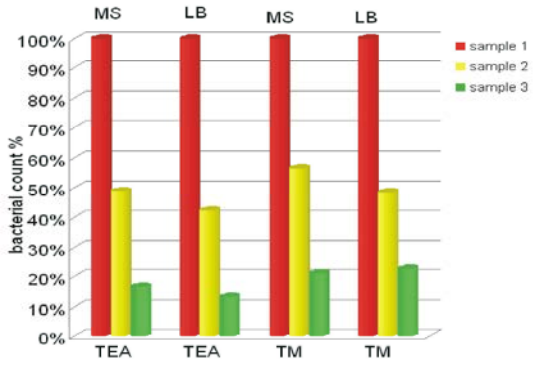

Fig. 1: The effect of tea and TM beverages drinking on bacterial counts % in saliva samples.

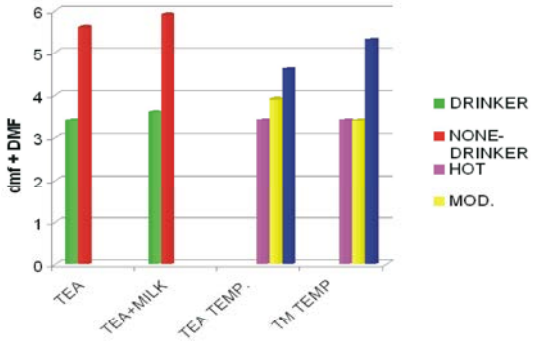

Fig. 2: The effects of beverage drinking and temperature on caries indices scores.

The bacterial counts and mean dmf+DMF scores were greatly lower ($P< 0.05$) in both beverages drinker groups on comparing with the non-drinker groups as shown in Table 2 and Fig. 2. Moreover, the table indicated that drinking hot TM could slightly decrease bacterial counts and dmf+DMF scores. However, beverage temperature had insignificant effects on *S. mutans* and *lactobacillus* counts as shown in Table 2.

Data in Table 3 indicated that there insignificant differences were observed among CFU/mL values of *S. mutans* and *lactobacillus* in relation to beverage consumption years number. Concerning the relation between the total dmf+DMF scores and drinking years, it is clear that there was a significant reduction of dmf+DMF scores ($P<0.05$) with the increase of beverage consumption years.

In regard to number of beverage cups and sugar spoons, negligible differences were detected among the initial bacterial counts of the classified groups as shown in Table 4. It is meritorious to know that the number of sugar spoons was directly proportionally with dmf+DMF scores.

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 [18, 29, 36].

Black tea and TM beverages showed various degrees of bacterial inhibition in post drinking samples but tea showed a higher bacterial reduction than TM. This may be referred to its high content of fluoride and polyphenolic compounds which are bactericidal and bacteriostatic. These results are in agreement with many studies [15-18] especially Abd allh *et al*. [22] who reported high reduction of oral SM (99.9%) and LB (98%) counts post tea drinking. In the present study, the relatively lower bacterial reduction of TM beverage may be due to formation of insoluble complexes between milk proteins and/or fat with tea polyphenols especially catechins, consequently reduce the bioavailability and accessibility of the polyphenols. This result is in agreement with many studies [38-40] and Van *et al*. [50] who reported that not the protein content itself is the cause of the inhibition but possibly the fat content.

The bacterial counts and mean dmf+DMF scores were lower in children drinking beverage than none drinking groups. These results are similar to previous clinical studies [22, 45, 51], which indicated significantly lower caries and plaque scores in tea drinker individuals (1-3 cups/day) than none drinkers individuals. There are convincing evidences that the bioactive components of tea and milk 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 [6, 44, 47]. Moreover, a significant inverse relation was observed between dmf+DMF scores and years number of beverage consumption. Since, the mean dmf+DMF scores were reduced (from 4.4 to 2.9 and 3.3 to 1.7) with increase the years of tea and TM consumption, respectively.

Regarding tea temperature, the present results showed that hot beverages reduce the bacterial counts and dmf+DMF scores by different rates when compared with moderate beverage temperature. This reduction in bacterial counts may be referred to rise of the oral tissues temperature that can affect oral microorganism's development and growth. At the same time, this rise in the tissues temperature lead to vasodilation of its blood supply; consequently increase of the blood flow and enhance the tissues immunity. De Jong *et al*. [52]

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 beverage cups and sugar spoons on the initial bacterial counts and dmf+DMF scores. The moderate consumption of tea and TM (2 cups/day) exhibited lower bacterial counts and dmf+DMF scores than groups given 1 cup/day. These results are in agreement with Abd Allah *et al*. [22] who reported that there is a significant inverse correlation found between amounts of tea drunk daily and DMF score. They attributed this effect to repeated fluoride intake.

It can be concluded that, black tea and TM beverages exhibited strong antimicrobial effect against *S. mutans* and *lactobacillus* bacteria. Also, the moderate consumption of these beverages (2 cups/day) exhibited low values of bacterial counts. In addition it showed significantly lower dmf+DMF scores in beverage drinker children. So it is recommended that the moderate intake of tea and TM are an effective natural measure to combat dental caries.

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