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Original Article

Effect of Non-Nutritive Sweeteners on Antibacterial Activity of Black and Green Tea Aqueous Extracts against Salivary *Mutans Streptococci* (*in-vitro* Study)

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Abstract

Herbal medicines, such as plants and their constituents, have been used globally to treat and cure disorders since antiquity, long before the discovery of modern drugs. Some of these items require an addition to make them more appealing to consumers. This study is an in vitro evaluation of the antibacterial activity of tea (black and green tea aqueous extracts) against salivary *Mutans streptococci*, followed by an analysis of the effect of non-nutritive sweeteners on the antibacterial activity of these extracts against salivary *Mutans streptococci*. The examined bacteria were sensitive to various doses of black and green tea aqueous extract, with the inhibition zone expanding as the concentration of the extracts rose. At a dosage of 225mg/ml for black tea extracts and 200mg/ml for green tea extracts, all *Mutans* isolates were destroyed. In this trial, 1% stevia or sucralose did not inhibit the antibacterial activity of any tea extract, nor did 5% stevia inhibit the antimicrobial activity of black tea extract. In addition, this concentration inhibits the antimicrobial properties of green tea extracts. In this investigation, it found that increasing the content of nonnutritive sweeteners interfered with the antibacterial activity of black and green tea aqueous extract against salivary *Mutans streptococci*.

Keywords: Dental Caries, Tea Extracts, Mutans Streptococci, Stevia, Sucralose

1. Introduction

Oral hygiene is essential for reducing the accumulation of dental plaque, a film of germs and food that forms on teeth (1). Mechanical aids such as toothbrushes, floss, and interdental cleaners include oral hygiene procedures. Chemical aids such as mouthwashes, dentifrices, and chewing gum assist preserve oral health, providing a risk-free and effective technique to reduce or remove plaque accumulation (2). In dentistry, the therapeutic effects of herbal drugs have been widely recognised, and their usage has gained widespread acceptance (3). Currently, mouthwashes containing plant components or extracts are gaining popularity because to their antibacterial plaque agents, anti-inflammatory properties, and pain-relieving properties (4).

In addition, herbal medication reduces microbial plaque efficiently in gingivitis and periodontitis (5). After water, tea is the second most consumed beverage. Tea refers only to non-alcoholic, caffeinated beverages produced by the infusion of the Camellia sinensis plant native to China (6). Numerous beneficial compounds have been discovered in tea and its preparation. Polyphenols, Pigments, Tea polysaccharides, Alkaloids, Amino Acids, and Saponins are among the most bioactive components. Numerous researches have demonstrated the antibacterial properties of tea. The majority of the direct effects of tea catechins arise from

catechins binding to the lipid bilayer cell membrane of bacteria, which then causes membrane damage (7).

Non-nutritive or non-caloric sweeteners are "food additives" that produce a sweet taste comparable to that of sugar yet containing less calories than sugar-based sweeteners (8). They are created by the production of plant components or chemical synthesis. They are also used to substitute carbs in diabetics' diets and to reduce the risk of dental cavities in chewing gum and sweets (9). Stevia (*Stevia rebaudiana* Bertoni), an Asteraceae perennial plant, is native to South America. Nevertheless, the plant is cultivated throughout, particularly in the Middle East (10). Due to the presence of numerous Steviol glycosides in its leaves, its sweetening power is 200–300 times that of sucrose, making it a suitable sugar alternative for use in the food and pharmaceutical industries (11). Besides glycosides, stevia is rich in essential amino acids, fatty acids, and phytochemicals (flavonoids, alkaloids, chlorophyll, xanthophyll, aromatic acid, chlorogenic acid, oligosaccharides, free sugars, necessary amino acids, and phytosterol). In addition, this plant is rich in vitamins such as niacin, thiamine, and ascorbic acid (12). Sucralose, a non-caloric sweetener authorised for use in foods and beverages and marketed under the brand name Splenda, is the most widely used artificial sweetener in the world. Sucralose's typical sweetness intensity is believed to be 600 times that of sugar (13). In the human body, the mouth cavity is a very diversified and fragile ecology. On occasion, conditions might alter, resulting in a disruption in the typical commensal connection between the host and resident oral microbiota, which tips the balance toward an increased risk of oral illnesses, most often dental caries and periodontal disorders (14). Mutans streptococci satisfy every condition for being a caries-inducing bacterial group; persons strongly colonised by mutans streptococci may be at a higher risk for caries (15). The purpose of this investigation was to determine the impact of non-nutritive sweeteners on the antibacterial activity of tea (black and green) aqueous extracts against salivary *Mutans streptococci*.

2. Materials and Methods

2.1. Study Design Sampling and Culture Methods

According to a study conducted by Thylstrup and Fejerskov (16), stimulated saliva samples were taken under conventional circumstances to obtain microbiological samples. Twenty healthy dental students between the ages of 18 and 22 participated in this research. Each participant was given 0.5 g of Arabic chewing gum and instructed to chew it for five minutes to stimulate saliva production. The saliva was then homogenised with a vortex mixer for two minutes. Following this, tenfold serial dilutions were made using phosphate buffer saline according to Beighton's procedure (17). 0.1 ml of dilutions 10-3 and 10-5 were extracted and spread in triplicate on Mitis Salivarius bacitracin agar, a selective medium for the isolation of *Mutans streptococci*. A single colony of *Mutans streptococci* was added to 10 ml of sterile Brain Heart Infusion broth (BHI-B) and cultured at 37C for 24 hours to create an active inoculum. In addition, to identify the microorganisms, Gram's stain, motility test, catalase test, and mannitol fermentation test were performed on the isolates.

According to Cowan, 100 g of black and green tea dry leaves were individually soaked in 500 ml of boiling distilled water, allowed to cool, and then stored for 24 hours to make the aqueous tea extracts (17). The infusion was filtered using Wattman No. 1 filter paper, and the remainder was discarded. The infusion was allowed to dry at room temperature in a glass Petri plate. A fine powder generated from the reaction was collected and kept in a dark glass container at room temperature until it was required to create different concentrations. The 50 mg/ml, 100 mg/ml, 200 mg/ml, 300 mg/ml, and 500 mg/ml concentrations of the extract powder were made with deionized sterile distilled water. Alternatively, two types of non-nutritive sweeteners were available on the local market: stevia in the form of powder and sucralose in the form of an aqueous compound with a 12.5 percent concentration. Final concentrations of stevia were prepared in 1 percent, 5 percent, and 10 percent by dissolving the

powder in distilled water, and final concentrations of sucralose were prepared from the stock compound after dilution in distilled water were 1 percent, 2 percent, and 3 percent Using the Agar well diffusion technique, the antibacterial activities of both types of tea extracts and the antibacterial potential of stevia and sucralose were evaluated against isolates dispersed on Brain Heart Infusion Agar (BHI-A). The density of activated microbial inoculum was adjusted to that of the standard turbidity (0.5 for bacterial isolates) McFarland standard turbidity to approach microbial cell density (1.5×10^8CFU/ml) by adding additional microorganisms or more sterile saline. Kork porer 6mm was used to make wells of uniform size and depth in the agar. Each well received 100 l of the extract, and distilled water served as the control.

Additionally, the plates were incubated for 24 hours in an aerobic atmosphere at 37 degrees Celsius. The minimum bactericidal concentration of black and green tea extract was determined using the agar streaking method; Different concentrations of tea extract were prepared by tube dilution method in BHI- B, inoculated with 0.1 ml of fresh microbial inoculum, and then incubated aerobically for 24 hours. Then, using a sterile microbiological lobe, streak it on BHI-A after dipping it in various extract concentrations. Therefore, the bacteria were eradicated by MBC, the lowest concentration of tea extract possible. Each Petri plate was incubated for 24 hours. Then, at 37 °C, the control plates (negative control, BHI-A streaked with microbial inoculums without the addition of the extract) and the positive control plates were placed in the incubator (BHI-A streaked with different concentrations of tea extracts without microbial inoculums). In each petri dish, microbial proliferation was noted. In addition, following the determination of the MBC value for black and green tea against the test microbes, the previously mentioned concentrations of stevia and sucralose were added to the MBC in accordance with Al-Mizrakchi (17) in order to determine the effect of adding non-nutritive sweeteners on the antibacterial activity of black and green tea aqueous extracts against salivary Mutans streptococci.

2.2. Statically Analysis

The data were analysed using the General Linear Model (Univariant Factorial ANOVA) and Tukey's Honestly Significant Difference (HSD) post hoc tests. Minimum, maximum, mean, and standard deviation were used to depict the data (SD).

3. Results

Mutans streptococci isolates were shown to be sensitive to the aqueous extracts of black and green tea, with the width of the inhibition zone increasing with increasing concentrations of the extracts. While the same microbiological isolates were not susceptible to either the aqueous preparation of stevia or the aqueous compound of sucralose, they were sensitive to the aqueous preparation of stevia (Tables 1-3 and Figure 1).

Table 1. Descriptive and statistical test of the diameter of inhibition zone against Mutans streptococci between the concentrations of each extract (10 isolates)

Extract	Concentration mg/ml)	Minimum	Maximum	Mean	±SD	F	Effect size	*P*-value*
Black tea	50	8.000	9.000	8.500	0.333	1446.448	0.985	0.000
	100	11.000	12.000	11.550	0.369			
	200	15.000	16.500	15.750	0.486			
	300	17.000	18.000	17.550	0.438			
	500	21.500	23.000	22.500	0.527			
Green tea	50	11.000	12.000	11.450	0.438	955.812	0.977	0.000
	100	14.000	15.500	14.550	0.497			
	200	16.000	17.000	16.400	0.394			
	300	18.000	19.000	18.450	0.438			
	500	22.500	24.000	23.200	0.537			

*=significant at $P<0.05$

Table 2. Multiple Comparisons between concentrations of the tea extract against Mutans streptococci using Tukey Honestly Significant Difference (Tukey HSD). (10 isolates)

Concentration	Conc.	Extract			
		Black tea		Green tea	
		MD	P-value*	MD	*P*-value*
50	100	-3.050	0.00000	-3.100	0.00000
	200	-7.250	0.00000	-4.950	0.00000
	300	-9.050	0.00000	-7.000	0.00000
	500	-14.000	0.00000	-11.750	0.00000
100	200	-4.200	0.00000	-1.850	0.00000
	300	-6.000	0.00000	-3.900	0.00000
	500	-10.950	0.00000	-8.650	0.00000
200	300	-1.800	0.00000	-2.050	0.00000
	500	-6.750	0.00000	-6.800	0.00000
300	500	-4.950	0.00000	-4.750	0.00000

*=significant at $P<0.05$

Table 3. Descriptive and statistical test of the diameter of inhibition zone of Mutans streptococci between extracts concentrations. (10 isolates)

Concentration	Extract	Minimum	Maximum	Mean	±SD	F	*P*-value*
50	Black tea	8.000	9.000	8.500	0.333	214.582	0.000
	Green tea	11.000	12.000	11.450	0.438		
100	Black tea	11.000	12.000	11.550	0.369	221.918	0.000
	Green tea	14.000	15.500	14.550	0.497		
200	Black tea	15.000	16.500	15.750	0.486	10.418	0.002
	Green tea	16.000	17.000	16.400	0.394		
300	Black tea	17.000	18.000	17.550	0.438	19.973	0.000
	Green tea	18.000	19.000	18.450	0.438		
500	Black tea	21.500	23.000	22.500	0.527	12.082	0.001
	Green tea	22.500	24.000	23.200	0.537		

*=significant at $P<0.05$

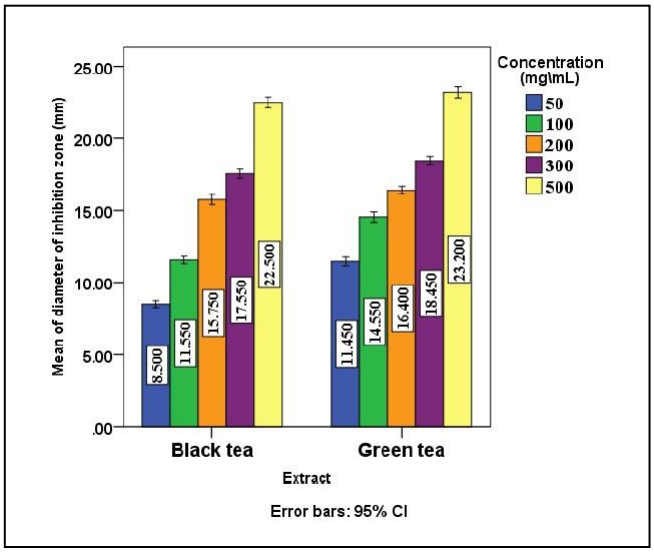

Figure 1. Comparison of the antibacterial activity (inhibition zone diameter in mm) of black and green tea aqueous extracts against Mutans streptococci

The minimal bactericidal concentration of black tea aqueous extract against *Mutans streptococci* in this experiment was 225 mg/ml. The MBC for green tea aqueous extract was 200 mg/ml against *Mutans streptococci* (Figure 2). In addition, adding 1% Stevia or sucralose to the MBC of the experimental extracts had no effect on the antibacterial activity of these extracts. Adding 5 percent to the MBC of black tea had no effect on the antibacterial activity of aqueous extracts of black tea against *Mutans streptococci*. In contrast, adding 5 percent Stevia to green tea aqueous extract diminished the extract's antibacterial effectiveness. In addition, the antibacterial activity of both tea extracts was impaired when the sucralose content exceeded 1 percent.

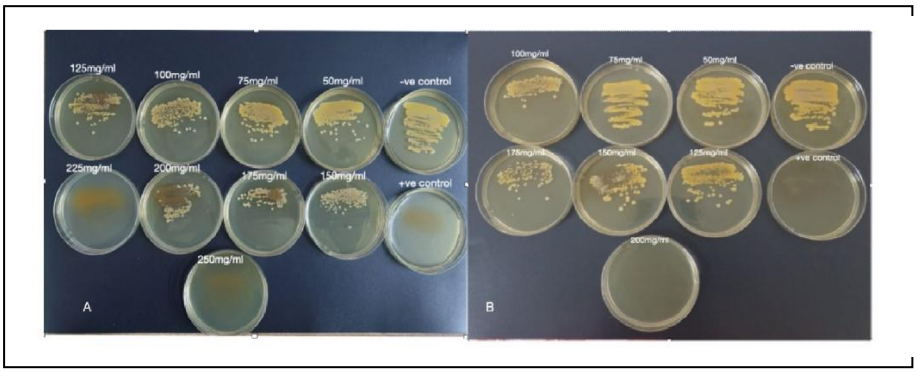

Figure 2. The minimal bactericidal concentration of **A:** Black tea **B:** Green tea aqueous extract against Mutans streptococci

4. Discussion

Oral illnesses are among the most widespread, impose considerable health and economic expenses, and considerably diminish the quality of life of people affected (18). The most widespread and consequential oral disorders globally are dental caries and periodontal disease (19). Scientists have been compelled to seek novel antibacterial compounds from many sources, including medicinal plants (20). Tea is a natural source of antibacterial compounds. Previous studies revealed the anti-cariogenic, anti-fungal, and anti-inflammatory properties of tea extracts in relation to oral infections (21). Mutans streptococci are a type of bacteria suspected of being the agent responsible for the onset and spread of dental caries (1). Therefore, the antibacterial activity of tea extracts against these microorganisms was examined in this study. This experiment utilised two varieties of dried tea leaves: black tea, representing fermented tea, and green tea, representing unfermented tea (6). Both black and green tea originate from a single plant (both C. Sinensis L.). Their changes in processing led to variations in chemical makeup, colour, and flavour (22). This study investigated the effect of aqueous tea extracts on the development of Mutans streptococci, as tea is typically supplied as an aqueous infusion (tea infusion). The bitterness test is the issue with employing tea as a mouth care ingredient in dental goods. Therefore, it typically requires reinforcement to become more acceptable to users. Therefore, a non-nutritive sweetener (NNS) was introduced to this trial. The addition of stevia and sucralose in quantities up to 1 percent had no influence on the antibacterial activity of experimental extracts against Mutans streptococci, which were killed at the same minimum bactericidal concentration (MBC) of the extracts before the addition of NNS. Possibly, there were no significant interactions between the NNS at up to 1 percent concentration and the extracts. This might have an effect on the important antimicrobial compounds included in these extracts,

including total polyphenols, catechins, tannins, flavonoids, and other chemicals. This finding is agreed with Korir, Wachira (23). They investigated the effect of adding NNS on the total phenolic components in tea and found that adding stevia at a concentration of 0.1g per 100ml of green and black tea aqueous extract had no significant effect. It Also agreed with Shalaby, Mahmoud (24), who proposed a mechanism for the influence of sweeteners on the radical scavenging activity of phenolic compounds in black and green tea and demonstrated that there were no significant interactions between aspartame glycosides and phenolic components in tea samples. Also, the addition of 5% stevia to black tea aqueous extract did not affect the antibacterial activity of that extract against *Mutans streptococci*, whereas the addition of 5% stevia interfered with the antibacterial activity of green tea aqueous extract; this may be due to differences in the total polyphenols (the primary antimicrobial agents) between black and green tea as a result of processing (6).

The bactericidal activity of black and green tea extracts was inhibited, however, by sucralose concentrations exceeding 1 percent. The precise mechanism through which NNS in high concentrations might inhibit the antibacterial action of extracts is difficult to determine at this time. It is hypothesised that the extracts' bioactive effects derive mostly from their ionised state, which can inhibit microbial development (25). In this instance, NNS may react with the functional sites of phenolic compounds to completely or partially inhibit their bioactive properties (26). The active redox reactions between the ionised hydroxyl groups of tea catechin (5,7,3',4'- tetrahydroxy -flavan-3) and those of sucralose may account for the interaction between black and green tea extracts as a rich source of flavonoids and sucralose, according to a separate study. Some additional compounds, such as pentagalloylglucose, tetragalloylglucose, and trigalloylglucose, may also be formed between NNS and gallic acids of the extracts during the formation of glucose hydroxyl groups that result in the formation of glucose-gallic complexes (27).

Additionally, the stevia component may contain ascorbic acid (AA) (28), which may interact with the polyphenolic molecule present in the extracts. AA possesses antioxidant properties and can be oxidised rapidly in aqueous solutions to form dehydroascorbic acid (DHAA) DHAA may capture catechin ascorbyl adducts EGCG, ECG, EC, and EGC. The capacity of DHAA to collect catechins ranged from high to poor for the four catechins EGCG, ECG, EC, and EGC. By generating ascorbyl adducts, DHAA might increase the degradation of catechins in tea, tea drinks, and tea meals (29).

Authors' Contribution

Study concept and design: D. M. A. and A. S. A.
Acquisition of data: D. M. A. and A. S. A.
Analysis and interpretation of data: D. M. A. and A. S. A.
Drafting of the manuscript: D. M. A. and A. S. A.
Critical revision of the manuscript for important intellectual content: D. M. A. and A. S. A.
Statistical analysis: D. M. A. and A. S. A.
Administrative, technical, and material support: D. M. A. and A. S. A.

Ethics

The study protocol was approved by the ethics committee of the University of Baghdad, Baghdad, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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