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A pilot study of the role of green tea use on oral health

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Abstract: *Introduction:* An increasing number of people all around the world are turning to the nature by using the natural herbal products in both prophylaxes and treatment of different diseases. Green tea with active chemical ingredients posses diverse pharmacological properties that include anti-inflammatory, anticariogenic, antioxidant and antibacterial effects. *Aims:* To assess the possible protective properties of green tea on oral health. *Methods:* The researchers used the following measurements: *Streptococcus mutans* count in saliva and plaque, Salivary and plaque pH values, Gingival Bleeding Index (GBI). The above-mentioned measurements were applied to a sample consists of 25 subjects before and after rinsing with green tea for 5 min (short-term study). While, *S. mutans* count for saliva and plaque and GBI measurements, this experimental intervention study was carried out in the El-Azhar University dental clinic. *Results:* The results of this study showed that there was a statistically significant difference among subjects pre- and post-rinsing with 2% green tea for 5 min concerning *S. mutans* count in saliva and plaque, salivary and plaque pH values and GBI. *Conclusion:* This study supports the effectiveness of local application of green tea as antibacterial and anticariogenic material as it decreases the acidity of the saliva and plaque, so it is a cost-effective caries prevention measures especially in developing countries.

Key words: dental caries; green tea; plaque; *Streptococcus mutans*

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Introduction

Dental caries are widely spread diseases affecting societies. Additionally, dental caries are one of the most important and widely spread dental diseases all around the world which defined as the breakdown ore softening of the enamel than

dentin (decalcification) layers and this destructive process progress more rapidly in dentin than in enamel to create tooth undermining or cavity (1).

Streptococcus mutans (*S. mutans*) has been identified as a plaque-forming bacteria, capable of producing dental caries in experimental animals and humans. The ability of *S. mutans* to adhere firmly to tooth surfaces in the presence of sucrose and to release acids fermenting various dietary sugars has been associated with its caries inducing potential (2). *Streptococcus mutans* infection has been implicated in dental caries and plaque formation. Several reports have demonstrated the efficacy of certain herbs against Mutans streptococci in both *in vitro* and *in vivo* studies (3).

Many attempts have been made to eliminate *S. mutans* from the oral flora, antibiotics such as penicillin; ampicillin, tetracycline, erythromycin and vancomycin are very effective in preventing dental caries *in vivo* and *in vitro*. However, their excessive use can result in alterations of the oral and intestinal flora and cause undesirable side-effects such as development of bacteria to tolerance, vomiting, diarrhoea and teeth stains (4).

Tea is an infusion of the leaves of the *Camellia sinensis* plant, green tea is one of the most widely consumed beverages in the world. Bioactive compounds in tea: polyphenols constitute the most interesting group of green tea leaf components, and in consequence, green tea can be considered an important dietary source of polyphenols, particularly flavonoids. The four major catechins are (−)-epigallocatechin-3-gallate, which represents approximately 59% of the total catechins; (−)-epigallocatechin (19% approximately); (−)-epicatechin-3-gallate (13.6% approximately) and (−)-epicatechin (6.4% approximately). Green tea also contains gallic acid and other phenolic acids such as chlorogenic acid and caffeic acid, and flavonols such as kaempferol, myricetin and quercetin. Apart from their polyphenol content, it is a natural source of fluoride and an effective vehicle for fluoride delivery to the oral cavity. After cleansing the mouth with tea, approximately 34% of the fluoride is retained and shows a strong binding ability to interact with the oral tissues and their surface integuments. This fluoride content may have a beneficial impact on caries (5).

Green tea is a very popular beverage, and *in vitro* studies have shown that green tea polyphenols inhibit the growth and cellular adherence of periodontal pathogens and their production of virulence factors (6). Green tea as natural plants was an inhibitor of the growth of *S. mutans* bacteria, which is a cariogenic bacteria, and this study concluded that, Gallocatechin was the most active component in inhibition of *S. mutans* and its minimum inhibitory concentration was around 250 mg ml^{-1} (7).

Rasheed and Haider (8) described the antibacterial effect of green tea catechins against *S. mutans* bacteria and stated that catechins were of great value in reduction of *S. mutans* bacteria and caries prevalence. Tsuchiya *et al.* (9) reported that green tea extract possess preventive effect against dental caries, and they concluded that when mouth rinsed with aqueous solution of the green tea extracts (5.0 mg ml^{-1}) contain catechins, the quantitative results revealed that catechins was retained in saliva for 60 min.

Lee *et al.* (10) concluded that catechins posses anti-plaque and antibacterial properties and suggested that green tea catechins contributed in caries prevention and gingival enhancement, and so holding green tea in the oral cavity for suitable time achieve locally high levels of catechins, which proved to be useful designing quality intervention to support anticariogenic, antibacterial and anti-plaque effects of green tea.

Adverse effect: harmful effects of tea over consumption (black or green) are due to three main factors: (i) its caffeine content, (ii) aluminium presence and (iii) the effects of tea polyphenols on iron bioavailability (5).

This study describes the possible antibacterial and anticariogenic properties of local application of green tea solution on the oral cavity.

Materials and subjects

This was a pilot study; samples were selected by systematic random sampling from patients arrived to dental clinic (El-Azhar University, Cairo, Egypt). Inclusion criteria were patient suffering from dental problems such as caries, gingivitis and/or periodontitis. Exclusion criteria from the study according to Park *et al.* (11) were patient received antibiotic therapy at last 3 weeks, had topical fluoride application at last 48 h, had topical mouth wash application at last 48 h and ate or drank at last 2 h before the study.

A total number of 25 patients were selected to participate in study according to the previous criteria, they were 13 males and 12 females, their age ranging from 21 to 46 years (mean 35.5 ± 3.6). The recruited subjects were given a brief explanation about the study and were instructed to give their informed consent to participate in the study. Every subject was asked about personal data and medical history (hypertension, diabetes and allergy).

Dental examination was carried out, then collection of saliva and plaque samples and analysis of the samples for estimation of pH and determination of *S. mutans* count were performed.

Dental examination

Full dental examination for all patients, using sterilized mirrors, probes and periodontal probes. Periodontal probe is the standard instrument for assessment of probing pocket; two conventional manual probes (Williams and WHO probes) were used in the present study (Orion Diagnostica Company, Espoo, Finland). Recording of caries prevalence according to Decayed, Missed and Filled (DMF) scoring system and gingival condition was examined and recording of Gingival Bleeding Index (GBI) was carried out (12).

Gingival Bleeding Index

Unwaxed dental floss was used then it passed interproximaly and curved around the tooth with denoting any bleeding point. Upper and lower anterior and premolar teeth in right and left sides used (18 interproximal areas). Gingival Bleeding Index = Number of bleeding areas/total number of scored proximal areas.

All patients were asked to:

1 Rinse with 10 ml of 10% sucrose solution for 2 min, then after 7 min saliva and plaque samples were taken (pre rinsing samples).

2 Rinse vigorously with water then after 1 h.

3 Rinse with 10 ml of 2% green tea solution for 5 min then after 20 min.

4 Rinse with 10 ml of 10% sucrose solution for 2 min then samples were taken after 7 min (post-rinsing samples). Experiments were designed according to Hirasawa *et al.* (13) (Fig. 1).

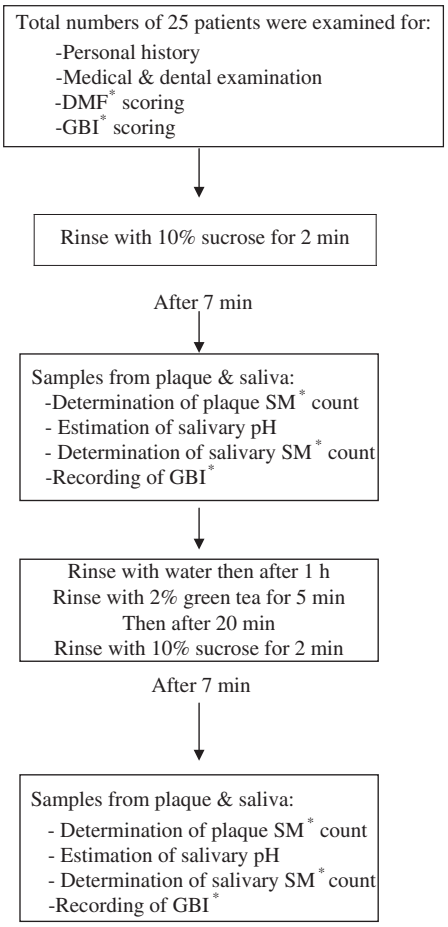

Fig. 1. Flow chart of the study.

Samples collection

1 Dental plaque samples: dental plaque samples 2–3 g were collected in sterile polypropylene tubes using sterile curettes or probes at the beginning before intervention and then at 3, 7, 11, 20 and 30 min intervals, then transferred to laboratory for further biochemical analysis.

2 Saliva samples: saliva samples (stimulated with paraffin wax) 4–5 ml were collected in sterile polypropylene containers for further biochemical analysis.

Samples stored in ice bags for 1–2 h till transferred to laboratory. All samples were collected and stored according to Wennerholm *et al.* (14).

3 Gingival condition was re-examined and GBI was recorded for detection of bleeding index after rinsing with green tea for detection of its effect on gingival bleeding tendency.

All previously collected samples were used for estimation of salivary and plaque pH, determination of *S. mutans* count in saliva and plaque.

Estimation of plaque and salivary pH

All samples of plaque were collected at different intervals, about 1 g was mixed with 1–2 ml of water, then pH was measured using digital pH meter; pH of all collected saliva was estimated directly by digital pH meter. According to Neta *et al.* (15) and Eliassan *et al.* (16), digital pH meter CG-820 (Schott-Gerate, Hofheim ATS, Germany) was used for estimation of plaque and salivary pH, the device was calibrated with standard buffer (Schott-Geräte GmbH-buffer solution, Hofheim, Germany) at pH 4.01 and 6.87, respectively, at 25°C and measurements were taken at 3 min as manufacture instruction.

Determination of plaque and salivary *Streptococcus mutans* count

Dentocult *S. mutans* strips (Orion Diagnostica) were used for determination of *S. mutans* count in both plaque and saliva (11, 17). According to manufacture instruction, bacitracin discs was added to culture vials, then after 15 min salivary samples dispersed on strips with round end and plaque samples speared on strips with square end then delivered into culture vials with quarter lock open and incubated at 37°C for 48 h. Bacterial count demonstrated from 0 to 3 as follow:

0 < 10 000 colony forming unit (CFU) ml^{-1}

1 < 100 000 CFU ml^{-1}

2 = 100 000–1 000 000 CFU ml^{-1}

3 > 1 000 000 CFU ml^{-1}

Statistical analysis

Using the SPSS Advanced Statistical Software version 13 (SPSS Inc., Chicago, IL, USA), the significance levels were determined between means at baseline (before rinsing with green tea) versus means after rinsing with it. Appropriate descriptive statistics and paired *t*-test used to determine significance.

Results

Table 1 compares between cases pre- and post-rinsing with green tea concerning SM count in saliva and it shows that there was a statistically significant difference ($P < 0.001$) pre rinsing (rinsing with 10 ml of 2% sucrose solution for 2 min only) and post-rinsing (rinsing with 10 ml of 2% green tea for 5 min then after 5 min rinsing with 10 ml of 10% sucrose solution for 2 min) *sign test*.

There were statistically significant differences ($P < 0.001$) between cases pre- and post-rinsing with green tea regarding SM count in plaque, *sign test* (Table 2) and in saliva *paired t-test* (Table 3).

Figure 2 shows that there was increase in plaque pH value, i.e. decrease acidity after rinsing with green tea at different time intervals (3, 7, 11, 20 and 30 min).

Table 4 shows that there was a marked reduction between cases pre- and post-rinsing with green tea concerning GBI score, this difference was statistically significant ($P < 0.001$) *sign test*.

Discussion

The possibility of preventing dental caries by the elimination of different pathogenic factors has been reported (18, 19). Investigation was focusing on the nutritional effects of tea, especially green tea on the human body, and there are suggestions that drinking green tea is an effective preventive method for caries to a considerable extent. The chemical composition of green tea and its content of different types of catechins exerts antimicrobial and antibacterial properties, moreover, it was found to have inhibitory effect on *S. mutans* growth, which are the main responsible for caries and gingivitis (7).

Catechins present in green tea represent marked effect on pH value of saliva and dental plaque concerning its reduction after eating towards acidic state and preserve it within normal range (11). Green tea extracts usage showed reduction in GBI because of its high content of catechins, so, oral application of catechins posses positive influence on the gingival and

Table 1. **Mean difference (pre- and post-rinsing with green tea) concerning *Streptococcus mutans* (SM) count in saliva**

SM count (mean ± SD)		Mean difference	SEM	95% Confidence intervals of the difference		*t*-value	d.f.	Significance
Pre rinsing	Post-rinsing			Lower	Upper			
1.72 ± 0.64	1.0 ± 0.76	0.72	0.092	0.531	0.909	7.856	24	$P < 0.001$

Table 2. **Mean difference (pre- and post-rinsing with green tea) concerning *Streptococcus mutans* (SM) count in plaque**

SM count (mean ± SD)		Mean difference	SEM	95% Confidence intervals of the difference		*t*-value	d.f.	Significance
Pre rinsing	Post-rinsing			Lower	upper			
1.68 ± 0.476	0.88 ± 0.526	0.80	0.115	0.562	1.038	6.928	24	$P < 0.001$

Table 3. **Mean difference (pre- and post-rinsing with green tea) concerning salivary pH**

Salivary pH (mean ± SD)		Mean difference	SEM	95% Confidence intervals of the difference		*t*-value	d.f.	Significance
Pre rinsing	Post-rinsing			Lower	Upper			
6.92 ± 0.201	6.43 ± 0.158	0.49	0.029	−0.42	−0.54	−16.475	24	$P < 0.001$

periodontal structures concerning gingivitis and periodontitis (20).

The present study investigated the effects of rinsing with 2% green tea solution for 5 min on *S. mutans* count in both saliva and plaque, salivary and plaque pH and GBI. The results showed that there was a statistically significant difference ($P < 0.001$) in salivary *S. mutans* count among patients rinsing with 10% sucrose solution only and those rinsing with 2% green tea then 10% sucrose. These results were in consequent with another study reported that rinsing with green tea (2%) was an inhibitor of the growth of salivary *S. mutans* bacteria with marked reduction in its count among cases before and after green tea application, it also proved that only 5 min exposure of cariogenic bacteria to green tea solution resulting in more than 50% decrease in CFU of *S. mutans* (7).

Otake *et al.* (21) studied the anti-caries effect of green tea polyphenols *in vivo* and they reported that polyphenolic compounds present in green tea posses high inhibitory effect against *S. mutans* bacteria growth and acid produced from it and that was the main cause of its anti-caries effect. Simonti *et al.* (22) stated that the increased activity of catechins present in green tea as anticariogenic material was related to barrier function of microorganism and depletion of thio group and they suggested that the inhibitory effect of rinsing with green tea on salivary *S. mutans* is questionable and in need for more work. The results of this study were in accordance with Elvin-Lewis *et al.* (23) that rinsing with green tea extracts naturally present had potential valuable anticariogenic activities including inhibition of *S. mutans* growth in saliva.

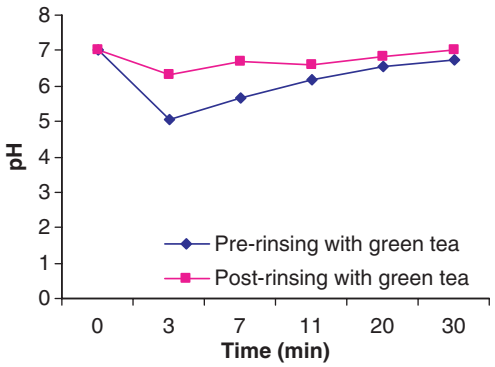

Fig. 2. Means of plaque pH at different intervals before and after rinsing with green tea.

Regarding the effect of green tea on *S. mutans* on dental plaque, there was a statistically significant difference among patients before and after rinsing with 2% green tea solution concerning *S. mutans* count in dental plaque. This result was in agreement with Sakanaka *et al.* (7), they revealed that rinsing with green tea extracts strongly inhibit the growth of *S. mutans* bacteria in plaque samples with decrease of its quantity. Zhong *et al.* (24) proved that *S. mutans* bacteria count in plaque showed descending levels after rinsing with 1% green tea solution for 5 min, Rasheed and Haider (8) described the antibacterial effect of green tea catechins against *S. mutans* in plaque samples and they discussed the great value of rinsing with green tea in inhibition of *S. mutans* growth in plaque and acid production.

The present study evaluated the effect of 2% green tea mouth wash *in vivo* and it concluded that there was a statistically significant difference ($P < 0.001$) before and after rinsing with green tea as regard *S. mutans* count in dental plaque. In contrary to this, another study carried out by Hirasawa *et al.* (13) concluded that green tea showed a little inhibitory effect on *S. mutans* count in plaque samples and marked inhibitory

Table 4. **Mean score difference (pre- and post-rinsing with green tea) concerning Gingival Bleeding Index (GBI)**

GBI (mean ± SD)		Mean difference	SEM	95% Confidence intervals of the difference		*t*-value	d.f.	Significance
Pre rinsing	Post-rinsing			Lower	Upper			
10.7 ± 5.4	5.08 ± 3.8	5.64	0.799	3.99	7.30	7.052	24	$P < 0.001$

effect on *S. mutans* count in saliva samples and acid production levels.

The result of this study demonstrated that there was a statistically significant difference ($P < 0.001$) among patients before and after rinsing with 2% green tea solution for 5 min concerning GBI and there was marked improvement in gingival colour and texture, this finding provides support for the postulate of Tsuchiya *et al.* (9) that rinsing with green tea solution showed significant difference in patients before and after rinsing concerning GBI, plaque index and gingival texture.

Kulik *et al.* (25) proved that local application of green tea extracts is of great value in enhancement of gingival and periodontal diseases especially when combined with mechanical scaling. Elvin-Lewis *et al.* (23) reported that green tea polyphenols is of prime importance in treatment of gingivitis. Catechins present in green tea posses antimicrobial effect against *S. mutans* bacteria the causative organism of caries and gingivitis and they recommended local application of green tea for caries prevention and GBI enhancement (26).

The results of this study were in disagreement with Beighton *et al.* (1999) (27), they studied the anticariogenic and antibacterial effects of green tea on 8 and 10 patients, respectively, with saliva and plaque samples analyzed 2–4 h later and they use 2% green tea concentration for 2 min only and they stated that green tea mouth wash showed minimal to no anticariogenic and antibacterial effects. This difference in result might be attributed to smaller sample size (8 and 10 patients) and shorter duration of green tea rinsing (2 min) while the current study analyzed saliva and plaque samples from 25 patients within 2 h before and after rinsing with green tea solution for 5 min.

This study revealed that there was a statistically significant difference among individuals before and after rinsing with green tea concerning plaque pH values at 3, 7, 11, 20 and 30 min and it proved that rinsing with 2% green tea solution for 5 min preserved plaque pH towards neutrality and prevented it from acidogenicity which is more suitable for growth of *S. mutans* bacteria and caries formation, these results were in accordance with another study revealed that plaque pH values decreased towards acidity after usage of sucrose only while it was preserved within normal values after rinsing with tea (15).

Hamilon-Miller (26) concluded that rinsing with green tea catechins for suitable time prevent acid production and preserve pH within normal range (7.2–7.4) which is not a favourable conditions for *S. mutans* growth so reduction in its quantity occurred and he stated that green tea posses anticariogenic and antibacterial properties.

Catechins present in green tea have marked effect on pH value of saliva and dental plaque and preserve it within normal range. Hirasawa *et al.* (13) evaluated the plaque pH value at different intervals 0, 3, 7, 11, 20 and 30 min among 15 subjects before and after rinsing with 2% green tea for 5 min and they proved that there was a significant difference among subjects concerning plaque pH values at 3, 7 and 11 min.

In the past few years, composite restoration become an alternative to amalgam fillings but recurrent caries around margins still represent major problem and more trials to reduce *S. mutans* count in oral cavity and around restorations will be of prime importance in increasing filling resistance and life time (28). The release of active ingredient from green tea (catechins) reduces plaque acidity and preserve pH towards neutrality which represented unfavourable medium for *S. mutans* growth and as the level of bacteria in saliva and plaque is related to caries development and secondary caries formation around composite restoration margins. Trials for reduction of *S. mutans* levels especially around composite restorations will be of great value in alteration of recurrent caries incidence (29).

Conclusion and recommendations

In order to have solid and generalizable recommendations, a study on adequate sample size should be conducted. This pilot study will be useful in alerting and initiating research activities on larger samples. The results of the present study proved that local application (oral rinsing) with green tea solution without sugar for short time strongly inhibit salivary and plaque *S. mutans* growth which are the main causative bacteria of caries both initial and secondary. It revealed a marked reduction in GBI score and enhancement of gingival condition. So, regular oral application of green tea solution in the form of mouth washes or its addition to dentifrices will be a cost effective caries prevention measures especially in developing countries.

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