ISSN : 2350-0743

www.ijramr.com

International Journal of Recent Advances in Multidisciplinary Research
Vol. 07, Issue 01, pp. 5567-5571, January, 2020

RESEARCH ARTICLE

ASSESSMENT OF THE ROLE OF GREEN TEA USE ON ORAL HEALTH

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ARTICLE INFO

Article History:
Received 24th October, 2019
Received in revised form
17th November, 2019
Accepted 05th December, 2019
Published online 31st January, 2020

Keywords:
Green tea, Dental Caries,
Streptococcus Mutans.

ABSTRACT

***Background and Aim*:** Streptococcus mutans **(**S. mutans**)** is the main etiological bacteria present in the oral cavity that leads to dental caries. Different preceding studies have recognized green tea to have anti-inflammatory, antiviral, antifungal, antioxidant, protein-denaturing, anti-mutagenic, anti-diabetic, anticarcinogenic, and antibacterial characteristics. The aim of our study is to investigate the effects of rinsing with 2% green tea solution for 5 min. on *S.mutans* count in both saliva and plaque and Gingival Bleeding Index (GBI). **Method:** Study was carried out in university hospital of Riyadh Elm University (REU) on 105 subjects. Each subject underwent two phases before and after rinsing with 2% green tea solution for 5 min. In each phase we measured GBI and *S.mutans* count in saliva and plaque. **Result:** The result of this study showed statistical significant differences between pre- and post- rinsing with green tea concerning *S.mutans* count in saliva and plaque and GBI. **Conclusion:** *S.mutans* count in both saliva and plaque is reduced after rinsing with 2% green tea solution for 5 min. and there is a marked reduction of GBI score.

INTRODUCTION

People around the world are using natural and herbal products for treatment of diseases. The products derived from medicinal plants are used for pharmaceuticals (Palombo, 2011; Ramesh, 2016). Green tea and its health benefits play a role in oral cavity. High molecular weight polyphenols are isolated from green tea possess antioxidant, antibacterial cariostatic, antitumor activities (Poonam Kumari, 2018). Dental caries pathogenesis involves several steps including the formation of a biofilm. A biofilm is defined as a community of bacteria that attach to a surface. While dental plaque is moderately specialized, it still shares the main properties of all biofilms. Biofilm formation is a three-stage process: docking, locking, and maturation (Blanco, 2005; Dunne, 2002). S.mutan **s**often gets the most attention in dental related studies because it has been previously shown to favor attachment to tooth enamel (Duchin, 1978; Kolenbrander, 2007). S. mutans infection has been implicated in dental caries and plaque formation (Al-Anbori, 2008). Dental caries is a multifactorial condition which is prevented by cleaning the teeth. Green tea extracts in dentifrices are used as an abrasive with strong antibacterial action (Sakanaka, 1989).

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Green tea is extracted from the leaves of Camellia sinensis**.** Camellia sinensis is shrub-like and is grown in a semi tropical environment on plantations in Southeast Asia. The active compounds in green tea are from a group of polyphenols called catechins. Four catechins present in green tea are: Epicatechin gallate (ECg), epicatechin, epigallocatechin and epigallocatechin-3-gallate (EGCG) (Anirban Chatterjee, 2012) The most active catechin, epigallocatechin-3-gallate (EGCG), makes up most of the content of the catechins at 59% (Taylor et al., 2015). Frequent intake of green tea can significantly decrease caries formation, even in the presence of sugar in the diet (Linke, 2003). Green tea extract also reduces α-amylase activity in saliva which makes it act as an anticariogenic agent (Zhang, 1998; Hirao et al., 2010). The aim of our study is to investigate the effects of rinsing with 2% green tea solution for 5 min. on *S.mutans*count in both saliva and plaque and Gingival bleeding index(GBI).

MATERIALS AND METHODS

Approval was obtained from Research Centre of REU. Study was carried out in university hospital of REU. The samples were selected by convenient sampling from patients attending outpatient clinic of Namuthajiyah and Olaya Campus of REU. Inclusion criteria were patients suffering from dental problems such as caries, gingivitis and / or periodontitis. Exclusion criteria from the study according to Park et al. (2006) were patients who received antibiotic therapy in the last 3 weeks,

had topical fluoride application in the last 48 h, had used mouth wash in the last 48 h and ate or drank 2 h before the study. A total number of 105 subjects (26 males and 79 females) ages ranging from 20 to 55 years qualified for the study. The recruited subjects were given a brief explanation about the study and were instructed to give their informed written 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 determination of S. mutans count.

Dental examination: Full mouth dental examination for all patients, using sterilized mirrors, explorer and periodontal probes. Periodontal probe is the standard instrument for assessment of probing pocket; two conventional manual probes CPITN and WHO probes were used in the present study. Recording of caries prevalence according to Decayed, Missing and Filled (DMF) scoring system and gingival condition was examined and recording of Gingival Bleeding Index (GBI) was carried out (Carter, 1974)

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:

- Rinse with 10 ml of 10% sucrose solution for 2 min, then after 7 min saliva and plaque samples were taken (pre-rinsing samples).
- Rinse vigorously with water then after 1 h.
- Rinse with 10 ml of 2% green tea solution for 5 min then after 20 min.
- 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.(2006)
- 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.

Determination of plaque and salivary *S.mutans*count: Dentocult S. mutans strips (Orion Diagnostica) (Figure 1) were used for determination of *S.mutans* count in both plaque and saliva (Park, 2006; Jensen, 1989). According to manufacture instruction, bacitracin discs was added to culture vials.Then subjects were asked to chew a paraffin tablet for five minutes in order to stimulate saliva and to transfer *S.mutans* from tooth surfaces into the saliva. 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 (Figure 2 and 3). Bacterial count demonstrated from 0 to 3 as follows (Figure 4).

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 16 (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 = 0.000). There were statistically significant differences (P = 0.000) between cases pre- and post-rinsing with green tea regarding SM count in plaque (Table 2). Table 3 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 = 0.000).

DISCUSSION

The results of our study demonstrated that there was a marked reduction of GBI among patients before and after rinsing with 2% green tea solution for 5 min and there was a statistically significant difference regarding *S.mutans* count in saliva and dental plaque. . This result agreed with Awadalla et al . they 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. They suggested that a study of adequate sample size should be conducted in order to have solid and generalizable recommendations (Awadalla, 2011). Sakanaka et al. revealed that rinsing with green tea extracts strongly inhibit the growth of *S.mutans* bacteria in plaque samples with decrease of its quantity (Sakanaka, 1989). Chatterjee A,et al.concluded that continuous use of green tea catechin on a daily basis may be a useful and practical method for the prevention of periodontal disease (Anirban Chatterjee, 2012). Nanri et al. investigated the relationship between the consumption of green tea and coffee and oral health-related quality of life(OHRQoL). OHRQoL was evaluated using the self-reported General Oral Health Assessment Index (GOHAI). they concluded that regardless of sex, green tea consumption was positively associated with the GOHAI score. Therefore, ≥3 cups/day of green tea may reduce the risk of a poor OHRQoL, especially in men (Nanri et al., 2018). In contrary to our result, another study carried out by Hirasawa et al. they reported that green tea showed a little inhibitory effect on *S.mutans* count in plaque samples and marked inhibitory effect on *S.mutans* count in saliva samples and acid production levels (Hirasawa, 2006). Melok, A.L, et al. established the first study that investigated EGCG-S as a potential anticariogenic agent. it suggested that EGCG-S can inhibit the growth of S.mutans**.** It is common for patients with infections of the oral cavity to be prescribed with 0.1% chlorhexidine gluconate. This comparative study was conducted over a period of 5 min and EGCG-S was effective in reducing bacterial growth at 1 min similar to the prescribed mouth wash chlorhexidine gluconate (Amy, 2018).

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

S. mutans Count in Saliva (Mean ± SD)		Paired Differences					Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95%Confidence Interval of the Difference		
Pre-rinsing	Post-rinsing				Lower	Upper	
1.6762±0.97562	0.6476±0.61988	1.0286	0.79005	0.07710	0.87568	1.18147	0.000

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

S. mutans Count in Plaque (Mean ± SD)		Paired Differences					Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95%Confidence Interval of the Difference		
Pre-rinsing	Post-rinsing				Lower	Upper	
1.7905±0.98737	0.8571±0.72627	0.9333	0.81177	0.07922	0.77624	1.09043	0.000

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

Gingival Bleeding Index (GBI) (Mean ± SD)		Paired Differences					Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95 % Confidence Interval of the Difference		
Pre-rinsing	Post-rinsing				Lower	Upper	
0.2795±0.16945	0.1968±0.14821	0.08272	0.09430	0.00920	0.06447	0.10097	0.000

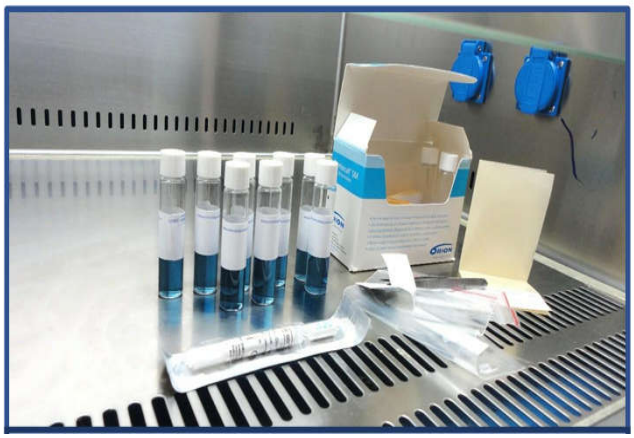

Figure 1. Dentocult S. mutans strips set

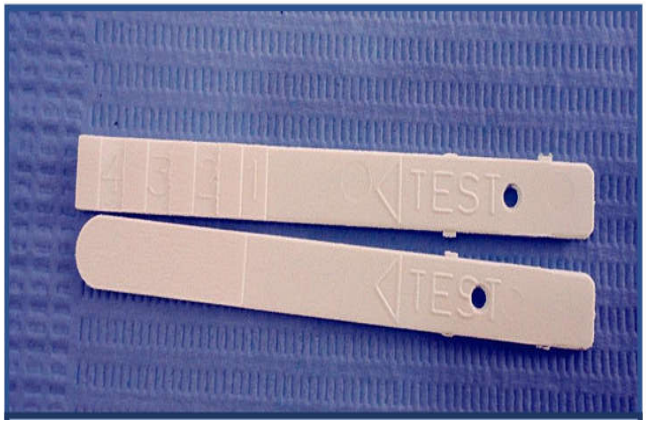

Figure 2. Saliva strip (round end) VS plaque strip (square end)

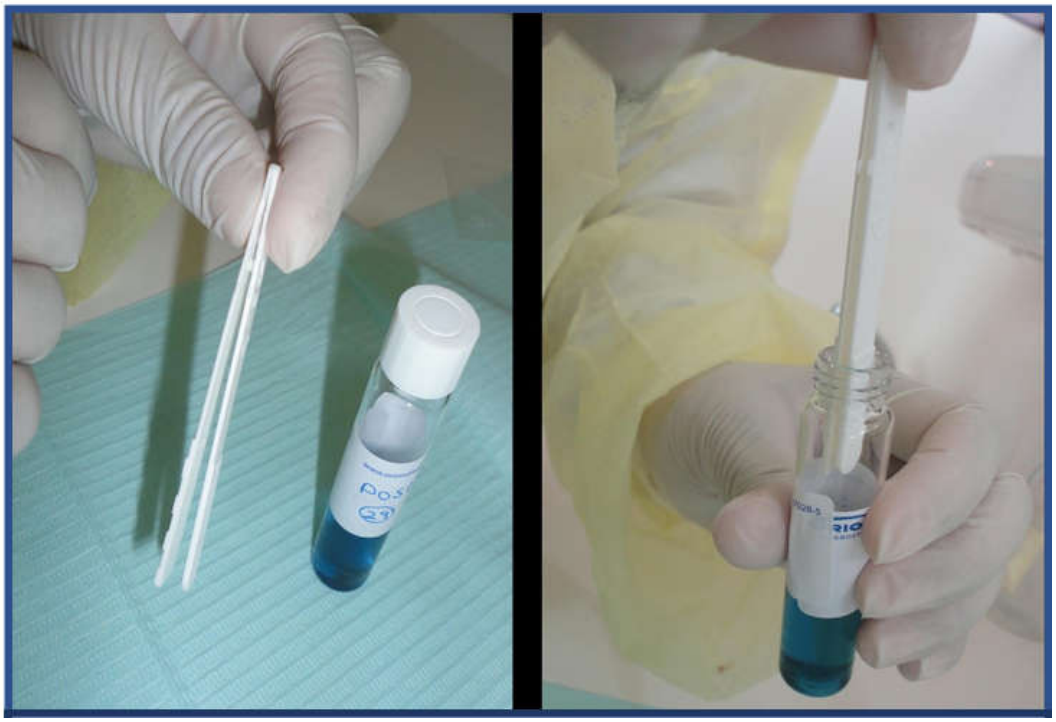

Figure 3. Place the used strips, attached back to back, in the selective culture

Figure 4. Pre-rinsing and post-rinsing samples formed colonies after incubation at 37°C for 48h

Kamalaksharappa SK, et al. assessed the efficacy of probiotic and green tea mouthrinse on salivary pH. they showed the beneficial effects of green tea in providing an alkaline environment which is conducive to the oral health of children (Kamalaksharappa, 2018). Abdelmegid F, et al studied the effect of green tea and honey solutions on the level of salivary*S.mutans*. They proved that a single time mouth rinsing with honey and green tea solutions for two minutes effectively reduced the number of salivary *S.mutans* of 7-10 years old boys (Abdelmegid et al., 2015). Green tea extract mouthwash is used in protection of erosion and abrasion of dentin of the teeth. Also reduces the virulent action of cariogenic pathogens like *Streptococcus mutans* and *lactobacilli*. Green tea extract reduces α-amylase activity in human saliva and inhibits the action of enzyme lactate dehydrogenase and reduces the acid production. Green tea powder reduces the volatile sulphur compounds and prevents halitosis. Green tea plays a role in maintaining oral health. Green tea reduces the incidence of dental caries through different mechanisms including enzymes activity and bacterial growth (Ramesh, 2016).

Conclusion

In the present study we concluded that rinsing with 2% green tea solution for 5 min. significantly reduced the *S.mutans* count in both saliva and plaque. It improved gingival status and decreased the Gingival bleeding index (GBI) score. So, this study is considered one of the recent research that study the benefits of natural ingredients for treatment and prevention of diseases. They are following the theory of returning to nature because natural products mostly safe and cost effective in comparison to other chemical medicines. At the end the green tea can be used as an alternative to chemical mouthwashes and can be added to dentifrices or chewing gums for caries prevention.

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