Evaluation of plaque and salivary pH changes after rinsing with refreshing beverages like basil tea and green tea: A cross-sectional study

Nagaraj Bkalburgi, Arati C Koregol, Ruchita S Patil, Tejashwini Puttarevanna, Pushpa Pattanshetty and Nandini Shiddaraj Shirigeri

Abstract

Growth of periodontal pathogens is affected by the changes in pH of dental plaque and saliva. An increasing number of people are using herbal products in both prophylaxis and treatment of various diseases. Green tea (GT) and Basil tea (BT) are healthy beverages consumed regularly which possess diverse pharmacological properties. The aim of our study is to evaluate the effect of rinsing GT and BT on dental plaque pH and salivary pH. Total of 140 subjects were selected and divided into 70 Healthy and 70 chronic periodontitis subjects. Each main group was subdivided into 35 rinsing BT and 35 rinsing GT. Plaque and saliva samples were collected and pH was analyzed by using digital Hannah pH meter before rinsing, at 15 & 30 mins after rinsing with BT and GT. Both BT and GT (intergroup pairwise comparison) showed statistically significant changes in salivary pH in chronic periodontitis subjects at both 15 and at 30 mins after rinsing with GT showing better results. Similarly, on plaque pH, BT showed better results than GT at both 15 and 30 mins after rinsing however, the statistically significant difference was seen only at 15 mins. Favorable changes in pH of plaque and saliva, conducive for periodontal health, occur after rinsing with BT & GT making it a safe and applicable adjunct in treatment of periodontitis.

Keywords: Basil tea; Green tea; pH; saliva; plaque; chronic periodontitis

1. Introduction

Periodontal disease is characterized by complex host-parasite interactions, changes in microbial composition, altered immune–inflammatory response and increase in oxidative stress which leads to gingival inflammation, loss of connective tissue attachment, periodontal ligament destruction and alveolar bone resorption. The primary etiological factor responsible for periodontal disease is “dental plaque” [1]. Plaque bacteria readily take up salivary calcium and phosphorous and use the minerals to protect them from the high pH, forming calculus which leads to periodontitis. The two key factors which are responsible for plaque formation are first, the presence oral bacteria to attack food particles and second the elevated pH. The pH must elevate above 7.6 to grow dental plaque crystals that cause periodontal disease [2]. Also, periodontal pathogens grow in a mildly acidic pH: 6.5–7 for P. gingivalis, 5.7 for P. intermedia and 5.5–7 for F. nucleatum [3, 4]. Variation in the pH can be seen in both saliva and plaque which ultimately affects periodontal tissues.

Saliva is an exocrine secretion that plays a very crucial role in oral environment and consists of nearly 99% water and 1% inorganic substances. Amount and characteristics of saliva have been associated with oral health. The average normal pH of saliva is 6.7, and in the oral cavity the pH is maintained near 6.7-7.3 by the buffering capacity of the saliva, which neutralizes acids produced by microorganisms and the acidity of foods and drinks. It has been demonstrated that individuals who have higher salivary level of inorganic calcium and phosphate, pH and flow rate and have poor oral hygiene would be at higher risk for developing periodontitis [5]. Diet is one of the important factors that affects the oral health and play a vital role among etiological for oral diseases. Nowadays people have become aware of the deleterious effects caused by carbonated beverages or packed fruit drinks and thus there is increasing trend towards more natural and healthy products [6]. Herbal tea like green tea, etc are nowadays consumed regularly and are presumed to play a vital role in maintaining oral health. Green tea is a non-fermented product of tea (Camellina sinensis) leaves that is consumed as a beverage worldwide. The active ingredients of green tea are polyphenols like flavonoids mainly catechins. Four major catechins includes Epigallocatechin-3-gallate (EGCG) (59%), epigallocatechin (EGC) (19%), epicatechin 3 gallate (ECG) (13.6%) and epicatechin (EC 4%) [7].

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Green tea extract is documented to have antibacterial action against *Porphyromonas gingivalis* and *Prevotella intermedia*, [8] antioxidant, [9] anti-inflammatory [10] and anticarcinogenic [11] properties. Green tea also has useful implications in oral health. Green tea mouthwash helps to reduce, plaque accumulation and gingival inflammation and halitosis [12] and an inverse relationship exists between the intake of green tea and progression of periodontitis [13]. Also, significant increase in salivary flow rate and pH after rinsing with green tea was seen in periodontitis group which concluded that green tea seems to be a safe and applicable adjunct treatment for periodontitis [8]. Tulsi (*Ocimum sanctum*) also called holy basil contains many nutrients and pharmacologically active compounds amongst which most important ones are essential oils and tannins. Primary components are eugenol 7%, methyl eugenol, a- caryophyllene, b- caryophyllene, carvacrol 3%, ursolic acid [14] flavonoids like orientin and vicenin, saponins and triterpenoids. Tulsi tea extracts possess antibacterial against *Actinobacillus actinomycetemcomitans* [15], *P. intermedia* and *F. nucleatum*, anti-inflammatory, antioxidant [16] analgesic and antipyretic properties. Holy basil mouthwash has showed inhibition of both periodontopathogens *P. intermedia* and *F. nucleatum* and has an antiplaque effect with prophylactic benefits [17].

To the best of our knowledge there is no enough scientific data available on salivary pH and plaque pH changes after rinsing with green tea and basil tea which may provide an insight on its role in the pathogenesis of periodontitis. In the view of this data and the possible positive effect of green tea and basil tea on the periodontal health, the aim of this study was to evaluate and compare the effects of green tea, basil tea on plaque pH and salivary pH and its effect on periodontium and thus evaluate its suitability as a diagnostic marker of disease also to evaluate the green tea and basil tea as a safe adjunct in the treatment of periodontitis.

2. Materials and methods

2.1. Study population

A total of 140 subjects aged 18–60 years were selected from the outpatient section of the Department of Periodontics, P.M. Nadagouda Memorial Dental College, Bagalkot, Karnataka, India. The protocol for this study was approved by the Ethics Committee of the institution. Duly signed written informed consent was obtained from all subjects participating in the study after the aims and procedures of the study was verbally explained to them. Data regarding the personal history, medical, dental, habit history was recorded. After screening, the patients were selected for the study.

Periodontal examinations were performed by a single and trained examiner. Gingival index (GI), simplified oral hygiene index (OHI-S), bleeding on probing (BOP), clinical attachment level (CAL) and probing pocket depth (PPD) were evaluated using Williams graduated probe. Based on the above mentioned criteria and type of tea given to rinse, subjects were divided into 4 groups, Group 1 included 35 Healthy subjects rinsing Basil Tea, Group 2 included 35 Healthy subjects rinsing Green Tea, Group 3 consisted of 35 Chronic Periodontitis subjects rinsing Basil Tea and Group 4 consisted of 35 Chronic Periodontitis Subjects rinsing Green Tea.

Patients with systemic diseases that could influence periodontal conditions, who have undergone periodontal therapy in the past 6 months, subjects on any drug therapy that can cause xerostomia, on systemic antibiotics, anti-inflammatory, hormonal therapy or corticosteroid therapy, immunosuppressive drugs for any other reasons which is known to affect the periodontal status, patients having tobacco in any form, pregnant or lactating mothers, patient with the history of radiotherapy and subjects with caries susceptibility were excluded from the study.

2.2. Green tea and (Tulsi) Basil tea preparation: Green tea and Basil tea were prepared by dipping of Green tea powder and basil tea powder infusion bag for 3-5 minutes in hot water of 80-90°C[9], 10ml of each green tea and basil tea were given for 60 secs to swish and spit [18].

2.3. Sample collection and processing: The subjects were asked to swallow immediately before plaque collection to minimize salivary contamination. Pooled supragingival plaque samples were collected using a sterile curette before rinsing, at 15 min and 30 min after rinsing with basil tea and Green tea. Each collected plaque sample was thoroughly mixed in a sterile container containing 5ml distilled water [19]. Subgingival plaque samples were collected from four deepest pockets in each quadrant and in healthy participants plaque samples were collected from mesial aspect of first four molars [20].

Unstimulated whole Saliva samples of 5 ml were collected in a sterile sample container. During saliva collection subjects were seated comfortably upright with eyes open, head tilted slightly forward and making minimal orofacial movements and saliva was allowed to accumulate in the floor of the mouth. The Subjects were instructed not to speak or swallow during collection. To minimize circadian influences, all the salivary samples were collected from 9 am to 11 am [2].

2.4. Determination of pH

The pH values for all saliva samples were assessed immediately after, before and 30 mins of rinsing with the help of Hannah pH meter (HI98107 pHeq®4 pH/Temperature Tester with 0.1 pH resolution). The pH meter was standardized using a standard protocol, using pH calibration solution of pH 7.

3. Statistical analysis

A sample size calculation was performed based on the data from Allen JC. Using these data, we estimated that we would require 80% power of 80% to detect a difference in periodontal conditions between the groups with a type I error at 5% and β error at 20%, thus giving a power to the study as 80%

4. Results

A total of 140 subjects were recruited under the study, with age ranging from 18-60 yrs, with mean age of 32.6 ±7.21 yrs in healthy subjects rinsing basil tea (group 1), 30.94±6.60

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yrs in healthy subjects rinsing green tea (group 2), 46.50±8.84 yrs in chronic periodontitis patients rinsing basil tea (group 3) and 46.94±10.79 yrs in chronic periodontitis subjects rinsing green tea (group 4). Intergroup age wise comparison showed statistically significant difference with highest mean age in group 4 and lowest mean age in group 2. Significant differences were observed in the statistical mean value of GI, OHI-S, BOP, PPD & CAL between healthy and chronic periodontitis patients as presented in table 1.

### Table 1: Intergroup comparison of all variables

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Chi-Square Value</th>
<th>P value of ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>32.6 ±7.21</td>
<td>30.94±6.60</td>
<td>46.50±8.84</td>
<td>46.94±10.79</td>
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<td>0.000**</td>
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<tr>
<td>GI</td>
<td>0.34±0.08</td>
<td>0.34±0.07</td>
<td>2.23±0.13</td>
<td>2.23±0.15</td>
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<td>98.19</td>
</tr>
<tr>
<td>OHI-S</td>
<td>0.70±0.12</td>
<td>0.68±0.09</td>
<td>4.56±0.69</td>
<td>4.43±0.72</td>
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<td>97.97</td>
</tr>
<tr>
<td>BOP</td>
<td>0.28±0.07</td>
<td>0.27±0.08</td>
<td>3.06±0.32</td>
<td>2.98±0.36</td>
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<td>98.44</td>
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<tr>
<td>PPD</td>
<td>1.15±0.44</td>
<td>1.18±0.39</td>
<td>7.33±0.36</td>
<td>7.29±0.42</td>
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<td>103.41</td>
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<tr>
<td>CAL</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>7.98±0.23</td>
<td>7.86±0.26</td>
<td></td>
<td>111.83</td>
</tr>
</tbody>
</table>

GI – Gingival Index, OHI-S – Oral hygiene index Simplified, BOP – Bleeding on probing, PPD – Pocket Probing depth, CAL – Clinical attachment loss.

For all tables and graphs,
** - statistically highly highly significant (p<0.01) * - statistically significant difference.
# - non-significant difference (p>0.05)
Group 1 – Healthy subjects rinsing Basil tea, Group 2 – Healthy subjects rinsing Green tea.
Group 3 - Chronic periodontitis subjects rinsing Basil tea.
Group 4 – chronic periodontitis subjects rinsing Green tea.

On intragroup comparison of salivary pH values, significant difference was seen in pH values before rinsing, at 15 mins, and at 30 mins after rinsing in healthy subjects rinsing with green tea (group 2), and chronic periodontitis subjects rinsing with both basil and green tea (group 3 & 4) as depicted in graph 1.

**Graph 1: Intragroup comparison of salivary pH**

Intragroup comparison of plaque pH values before rinsing, 15 and 30 mins after rinsing as depicted in table 4, for all the four groups, showed statistically significant difference in levels of plaque pH with higher values at baseline and least at 30 mins after rinsing as depicted in graph 2.

**Graph 2: Intragroup comparison of Plaque pH**

Intergroup comparison of salivary pH values showed statistically significant difference, at all the intervals, before, at 15 and 30 mins after rinsing with salivary pH values highest in healthy subjects rinsing basil at 15 mins (7.23±0.37) and 30 mins (7.31±0.21) and least in chronic periodontitis subjects rinsing basil tea at both 15 mins (6.66±0.60) and 30 mins (6.90±0.26) after rinsing as depicted in graph 3.

**Graph 3: Intergroup comparison of salivary pH**

On Intergroup comparison of plaque pH values statistically significant difference was seen, before rinsing and at 15 mins after rinsing. At 15 mins after rinsing lowest plaque values were seen in healthy (7.45±0.38) and chronic periodontitis subjects (7.60±0.49) rinsing with basil tea and higher values were observed in healthy (7.63±0.21) and chronic periodontitis subjects (7.68±0.37) rinsing green tea as depicted in graph 4.

**Graph 4: Intergroup comparison of plaque pH**

Intergroup pairwise comparison for salivary pH values was done showing statistically significant difference in chronic periodontitis subjects rinsing basil tea and green tea (p= 0.013* & p= 0.037*) at both 15 and 30 mins respectively after rinsing, with green tea showing better results at both 15 mins (pH= 6.8) and 30 mins (pH= 7.0) after rinsing. On intergroup
pairwise comparison for plaque pH in chronic periodontitis subjects, basil tea had more pronounced effect than green tea at both 15 mins (pH = 7.60) and 30 mins (pH= 7.35) after rinsing, however this difference was found to be statistically non-significant (p= 0.69#, 0.71*) at both 15 and 30 mins respectively after rinsing as depicted in table 2. Also, healthy subjects showed statistically significant difference in pH values at both 15 and 30 mins (p=0.00*) rinsing green tea whereas healthy subjects rinsing basil tea showed statistically non-significant difference.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group</th>
<th>Vs group</th>
<th>P value of Mann whitney test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salivary pH at 15 mins after rinsing</td>
<td>3</td>
<td>4</td>
<td>0.013#</td>
</tr>
<tr>
<td>Salivary pH at 30 mins after rinsing</td>
<td>3</td>
<td>4</td>
<td>0.037*</td>
</tr>
<tr>
<td>Plaque pH at 15 mins after rinsing</td>
<td>3</td>
<td>4</td>
<td>0.069#</td>
</tr>
<tr>
<td>Plaque pH at 30 mins after rinsing</td>
<td>3</td>
<td>4</td>
<td>0.714#</td>
</tr>
</tbody>
</table>

5. Discussion
In the past few years, an increased focus has been put 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 amongst which, basil tea and green tea are ones which shows anti-microbial, anti-inflammatory and antioxidant properties [18]. Basil tea contains active ingredients mainly in the form of essential oils and tannins. The fixed oil and linolenic acid possess significant anti-inflammatory activity against PGF2, leukotriene and arachidonic acid by virtue of their capacity to block both the cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism [21]. Tusli (Basil) extracts has shown anti-microbial activity against Actinobacillus actinomyctetcomiensis, [15] P.intermedia and F. nucleatum [17]. Antimicrobial agents in Ocimum sanctumare mainly in the form of essential oils, which contains monoterpene components that are mostly phenolic in nature. They exert membrane damaging effects and stimulate leakage of cellular potassium which is responsible for a lethal action related to cytoplasmic membrane damage [19].

Basil increases the anti-oxidant molecules such as glutathione and enhances the activity of anti-oxidant enzymes like superoxide dismutase and catalase, which protect the cells from free radicals. Also, it enables the body to transform and eliminate toxic chemicals by enhancing the activity of liver detoxification enzymes such as the cytochrome P450 enzymes, which deactivate them and enables them to be safely excreted [22].

Green tea is a rich source of flavonoids mainly catechins. The antibacterial effect is through the inhibitory effect of EGCG and EGC on cysteine proteases of P. gingivalis [23] & P. intermedia [24]. They also act as antioxidants through the induction of antioxidant enzymes such as glutathione S-transferase and superoxide dismutase. Also, catechins bind to iron and copper ions, thus reducing the impact of these ions on oxidation reactions. They also prevent the activation of redox-sensitive transcription factors, which are mediators of inflammatory reactions. Catechins can also suppress other oxidation substances, such as nitric oxide synthase, cyclooxygenase2 (COX-2), lipoxygenase-2 (LOX-2), and xanthine oxidase [25].

In our present study, statistically significant difference (p<0.001) was found in the salivary pH and plaque pH before and after rinsing with Basil tea and Green tea in chronic periodontitis subjects which was in accordance to the study conducted by M A Adil Ahmed et al. [18] which showed statistically significant increase in pH of saliva and plaque after intake of green tea and chamomile tea. Awadalla et al. [19] also observed significant increase in pH, causing decrease in the acidity of plaque and saliva after rinsing with 2% green tea. Similar changes were observed in salivary pH by Masoumi et al. [31], after consumption of green tea, in periodontitis group. Catechins present in green tea represents marked effect on pH value of saliva and dental plaque concern its reduction after eating towards acidic state and preserve it within normal range[10].

Hamilon-miller [26] concluded that rinsing with green tea catechins for suitable time reduces the acid production and preserves pH in normal range (7.2-7.4). This pH is unfavorable for growth of anaerobic organisms present in the dental plaque thus reducing the progression of periodontitis. Also, the reduction in oxidative stress [27, 28] due to antioxidative property of green tea catechins and basil tea were thought to decrease the acidity of salivary and plaque pH, which in turn causes unfavorable environment for growth of anaerobic organisms present in plaque. In our present study, healthy subjects showed significant difference in plaque pH after rinsing with green and basil tea but salivary pH changes were significant only after rinsing with green tea and not with basil tea. This was in contrast to study by Masoumi et al where no significant change in salivary pH was seen in healthy group after green tea consumption [5].

Few other studies like, by Shaila et al. [29]studied the salivary protein concentration in gingivitis and periodontitis patients and also compared parameters like salivary flow rate and pH showed that there were no significant changes in flow rate or pH between disease and control group subjects which contrasting to our results. Gazy et al. [30] evaluated some salivary biochemical parameters in patients with chronic periodontitis and normal subjects. They found no difference in salivary acidity level between the groups which is in contrast to our present results.

6. Conclusion
The results suggest that, on rinsing with Green tea and Basil tea causes significant changes in salivary pH and plaque pH with Green Tea showing better results in Salivary pH and Basil tea showing better results in plaque pH, which is conducive for periodontal health, its daily consumption helps to improve overall status of periodontal tissues by decreasing microbial load, inflammation and by increasing anti-oxidants and seems to be a safe and applicable adjunct treatment for periodontitis. Also, pH of plaque and saliva can be used as one of the diagnostic markers for periodontal diseases. However, changes in pH of plaque and saliva after rinsing with green tea and basil tea and its influence on periodontal status require further long-term investigations.

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References


