Different effect of green tea consumption on salivary antioxidant status in light versus heavy smokers

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Abstract

Purpose: This clinical trial study sixty volunteer healthy male smokers (light and heavy) and non-smokers were selected according to the inclusion criteria. Participants of each three groups were instructed to drink 4g of green tea (prepared with 300 ml hot water) daily, for three weeks. Total antioxidant capacity of saliva was measured at baseline, after 7 days, and after 21 days in each group. Repeated measure ANOVA with Bonferroni adjustment was performed for statistical analysis.

Results: Non-smokers had a higher amount of salivary total antioxidant capacity at baseline (p<0.001). After 7 days of green tea consumption total antioxidant capacity of non-smokers and light smokers showed no statistical difference (p=0.075), this trend continued until 21 days. In the heavy smokers total antioxidant capacity was still different from the other two groups (p<0.001). However, the maximum positive alteration of salivary total antioxidant capacity from day zero to day 21 occurred in the heavy smoker group (p< 0.001).

Conclusion: Although findings support the role of green tea drinking in reducing oxidative damage in saliva of both groups of smokers, heavy smokers showed the most significant change in total antioxidant capacity levels over three weeks.

Keywords: Antioxidant, Green tea, Saliva, Smokers

Introduction

Cigarette smoke contains high amounts of chemicals that lead to formation of oxidants and free radicals. It is estimated that each puff of cigarette has almost 1016 oxidative molecules. These free radicals and reactive oxygen species can damage DNA directly or indirectly via inflammatory processes. They play a significant role in the pathogenesis of many life-threatening diseases including oral cancer. In fact, consumption of tobacco, as well as alcohol consumption are the two major risk factors of head and neck cancer (HNC) with approximately 2/3 attributable to smoking and cumulative smoking exposure were associated with worse prognosis in some cancer patients. On the other hand, green tea (GT) is a beverage with large amounts of catechism, monomeric polyphenols, and has powerful antioxidant activity for scavenging reactive oxygen species (ROS). This antioxidative property lead to potential health benefits associated with GT consumption due to the preventive effect in ROS-related diseases such as cancers. Some epidemiologic evidence suggests that GT has chemo-preventive effects in cigarette smoke induced cancers. A recent case-control study concluded that tea drinking might decrease the risk of oral cavity cancer. However, they suggested further investigation for clarification of underlying mechanisms.

Saliva is the first line of defense against encountered cigarette smoke. It has direct contact with tea constituents. Previous studies showed tea catechins could be measured in saliva even after vigorously rinsing the mouth. Moreover, salivary antioxidant capacity is a good measurement for monitoring antioxidant alternation in smokers. In addition, the results of one study suggested salivary antioxidants are different in heavy smokers compared to light smokers and controls. However, investigations are limited and results are inconclusive.

Considering the safety and availability of GT over an extended period of time as a chemo preventive agent, and a lack of evidence on the salivary antioxidant capacity of smokers after consumption of GT, the aim of this study was to evaluate the effect of GT drinking (over a 3-week period) on the salivary total antioxidant capacity (TAC) in both light and heavy smokers.

Materials and Methods

The study was conducted according to the principles of the “Declaration of Helsinki”; and approved by the Ethics Committee of the Shahid Beheshti University of Medical Sciences (IR.SBMU.RIDS.REC.1394.76). All participants signed an informed consent document prior to the study. In this clinical trial study sixty volunteer healthy male cigarette smokers (CS) (light and heavy) and non-smokers (NS) were selected according to the inclusion criteria. For sample size calculation α=0.05, β=0.2 were selected for...
detecting 0.5 unit difference between the mean antioxidant change between groups with SD=1. Therefore 20 subjects should be enrolled for each of the studied groups. Current smokers who have ever smoked 100 cigarettes over their life and smoked daily in the past 30 days were categorized as light and heavy smokers. Light smokers should have reported less than 10 cigarettes per day and heavy smokers should have reported more than 10 cigarettes per day. Non-smokers were defined as those who self-reported never smoking. A convenience sampling method was followed, using volunteer participants who attended to the oral medicine department of Shahid Beheshti dental school. The studied groups were tried to be matched by age and gender. Participants were included in this study who had at least 20 teeth, with no history of any surgical or non-surgical periodontal therapy in the past 6 months; and did not drink more than one cup of green tea or 3 cups of black tea or coffee daily. Subjects with a history of any systemic diseases, regular users of mouthwash, medications or vitamin supplements within the past 3 months, those who had special dietary requirements, or alcohol and drug abusers were excluded from the study. All participants were examined for clearance from oral mucosal lesions before inclusion in the study. A questionnaire consisted of questions related to the demographic variables were filled for all of the participants. The duration of smoking, number of cigarettes per day, brand of cigarette (domestic or imported) and consumption of other types of tobacco were also asked from smoker groups.

Participants were asked to consume two cups of GT per day, between breakfast and lunch, and between lunch and dinner, for a period of three weeks. Every cup was prepared by infusing 2g of GT in 150 ml of hot water (80°C) for 3 minutes. We supplied the same brand of GT (best-known in the country) from same batch in small tea bags, after careful weighting with a digital scale. Also, we provided the same glasses to each participant for equalizing the amount of heated water. They could not add milk to the tea, but addition of sugar was allowed. The bags of tea were labeled with each participant’s number, and they were asked to stick their labels in a daily diary as a compliance check. Participants were instructed to adhere as closely as possible to their normal eating habits during the experiment. Moreover, they were not allowed to consume more than 2 oranges or 2 glasses of fruit juice per day or to drink more than one cup of black tea.

Saliva collection: Whole unstimulated saliva was collected at baseline, after 7 days, and after 21 days of tea consumption. Participants spat almost 2 ml of their saliva into falcon tubes in an upright position, between 9 am and 12 pm, after rinsing the mouth with 15 ml of distilled water. They had been requested to avoid eating, drinking or smoking at least 1 hour before the saliva collection. The samples were immediately centrifuged at 3,000 (rpm) at 4°C for 15 minutes; then was stored at -70°C until analysis. Total antioxidant capacity assay (TAC): TAC was measured by the ferric reducing ability of plasma (FRAP) method. This method is based on the ability of plasma to reduce Fe III to Fe II in the presence of TPTZ (tripyrtdyltriazine). After reaction, samples were read with a spectrophotometer at maximum absorbance in 593 nm. This method has previously been used for measuring salivary TAC. All samples were measured at the same time, and results were read with one calibrated spectrophotometer by one experienced technician who was blind about cases, under supervision of a clinical biochemistry specialist. Results are expressed as mean± standard deviation. One way ANOVA was done to compare the mean age between 3 groups. Type of cigarette and duration of smoking were compared between light and heavy CS groups by Chi-square and independent t-test. The differences between groups were assessed by repeated measures ANOVA and Bonferroni tests. We assumed a p-value of <0.05 as statistically significant. IBM SPSS Statistics for Windows, version 21.0 (IBM Corp., Armonk, N.Y., USA) was used for statistical analysis.

Results

Sixty males were participated in the present study. The age of the heavy CS group was 31.3±6.7, light CS group was 31.75±7.39 and NS group was 31.8±7.3. There was no statistical difference between the mean age of three groups (p=0.97). Two smoker groups were similar in terms of other type of tobacco consumption (p=0.76), domestic or imported cigarette brands (p=0.62). Duration of smoking was 6.25±3.54 and 6±6.43 years for heavy and light smokers, respectively (p=0.82). TAC levels at baseline were 338.8±60, 592.2±46.3 and 686.6±62.2 for heavy, light and non-smokers, respectively. Significant differences were found in mean TAC levels between groups at baseline (p<0.001). At day 7, there was no difference between light CS and NS (p=0.09). However, the difference of TAC between heavy CS and the two other groups was significant (p<0.001). Also, on day 21, there was not a significant difference between light CS and NS (p=0.098), but the difference of TAC between heavy CS and two other groups was significant (p<0.001). There was an upward trend in salivary TAC over the study period (baseline, day 7, day 21) (Figure 1). The positive change in the TAC was statistically significant for heavy CS group in all -time measurement points (p<0.001) (Table 1). For light CS a considerable increase was seen between baseline with 7th and 21 days (p<0.001), but there was not a significant difference between the mean TAC levels for day 7 and 21 (p=0.34). However for NS group there was not a significant difference in TAC levels between study periods.
Discussion

According to the results, there was an upward trend in salivary TAC of smoker groups over the study period (baseline, day 7, day 21) after consumption of 4g of GT. This amount of GT is in line with other studies and had no toxic effects. This increase in antioxidant levels in smokers due to the free radical scavenging action could protect cells from further cellular damage and in turn cancer prevention.

The effect of green tea on cigarette smoke-induced oxidative damage has been evaluated in previous studies. Schwartz et al (2005) in a pilot study in humans evaluated molecular and cellular effects of drinking five cups of GT per day, analyzing oral cells of heavy smokers. They reported that, during the course of GT administration, smoking-induced DNA damage decreased and cell growth was inhibited. Hakim et al. (2008) in an intervention trial evaluated the efficacy of regular GT drinking in reducing DNA damage as measured by urinary 8-hydroxydeoxyguanosine (8-OHdG) among heavy smokers, and concluded that a statistically significant 31% decrease in urinary 8-OH-dG occurred in smokers compared with the baseline.

In conclusion, results showed after 7 days of GT consumption, the salivary TAC levels in light smokers increased to levels near that of the NS group. Heavy smokers showed the most significant TAC alteration after 21 days, but even after this period, they had lower TAC levels.

Table 1 - Comparison of TAC levels between 3 groups in study periods.

<table>
<thead>
<tr>
<th>Group name</th>
<th>Baseline</th>
<th>Day 7</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>686.6±62.2</td>
<td>695.5±57.8</td>
<td>703.1±55.6</td>
</tr>
<tr>
<td>Light CS</td>
<td>595.2±46.3</td>
<td>648.1±60.4</td>
<td>656.8±63.2</td>
</tr>
<tr>
<td>Heavy CS</td>
<td>338.8±60.0</td>
<td>451.3±60.4</td>
<td>484.8±79.4</td>
</tr>
</tbody>
</table>

Conclusion

In conclusion, results showed after 7 days of GT consumption, the salivary TAC levels in light smokers increased to levels near that of the NS group. Heavy smokers showed the most significant TAC alteration after 21 days, but even after this period, they had lower TAC levels.
levels, compared to the other groups. Although, drinking of green tea could not change the smoking habits, these findings support the role of GT in reducing oxidative damage in saliva of smokers.

In this study we evaluated only TAC in saliva as an indicator of oxidative damage, however, evaluation of other marker of oxidative damage and compare them between saliva and plasma in different gender is suggested for future studies.

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Conflict of Interests

None Declared

References

Green tea and salivary antioxidant in smokers

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