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

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(Objective) Oxidative stress consequent to cigarette smoking may alter the salivary antioxidant defense system and lead to oral cancer. Green tea, with antioxidant properties, interacts with saliva upon entering the mouth. This experimental study explored the preventive effect of green tea on cigarette smoke-induced oxidative damage over 3 weeks.

Methods In 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.\textsuperscript{1} It is estimated that each puff of cigarette has almost 1016 oxidative molecules.\textsuperscript{2,3} These free radicals and reactive oxygen species can damage DNA directly or indirectly via inflammatory processes.\textsuperscript{2,4,5} They play a significant role in the pathogenesis of many life-threatening diseases including oral cancer.\textsuperscript{6} 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 contributions.\textsuperscript{6} Previous studies reported smoking status, quantity of cigarettes per day and cumulative smoking exposure were associated with worse prognosis in some cancer patients.\textsuperscript{7}

On the other hand, green tea (GT) is a beverage with large amounts of catechin, monomeric polyphenols, and has powerful antioxidant activity for scavenging reactive oxygen species (ROS).\textsuperscript{2,4,8} This antioxidant property lead to potential health benefits associated with GT consumption\textsuperscript{9} due to the preventive effect in ROS-related diseases such as cancers.\textsuperscript{10} Some epidemiologic evidence suggests that GT has chemo-protective 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.\textsuperscript{11}

Saliva is the first line of defense against encountered cigarette smoke\textsuperscript{12}. It has direct contact with tea constituents. Previous studies showed tea catechins could be measured in saliva even after vigorously rinsing the mouth.\textsuperscript{5,13} Moreover, salivary antioxidant capacity is a good measurement for monitoring antioxidant alternation in smokers.\textsuperscript{14} In addition, the results of one study suggested salivary antioxidants are different in heavy smokers compared to light smokers and controls.\textsuperscript{15} 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,\textsuperscript{16} 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 \(\alpha=0.05\), \(\beta=0.2\) were selected for...
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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 (tripyriddylietrizaine). 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

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±6.7. 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.
of oxidative stress\textsuperscript{20}. Narotzki et al. (2013) conducted a study in order to elucidate the interaction between GT and its main polyphenol EGCG (Epigallocatechin 3-gallate), and activity of oral peroxidases (OPO). They observed a rise of OPO activity following addition of GT and in a dose dependent manner. They concluded that tea consumers’ oral epithelium might provide an extra protection against the deleterious effects of hydroxyl radicals, produced by not removing hydrogen peroxides in the presence of metal ions\textsuperscript{20}. In an interventional, crossover trial in elderly subjects, Narotzki et al. (2014) reported saliva TAC was improved by 1.5g GT drinking. However, no changes were observed in saliva oral peroxidase enzymes\textsuperscript{27}. Studies reported the difference in salivary TAC in smokers and non-smokers\textsuperscript{12, 26}. Also, the results of a recent study advocated local compensatory mechanism in saliva due to increase in salivary total antioxidant capacity in patients with head and neck squamous cell carcinoma\textsuperscript{25}. In recent study, we evaluated the role of GT on improvement of salivary antioxidant capacity of smokers and results support the effectiveness of green tea drinking in salivary antioxidants enhancement in smokers after three week\textsuperscript{20}. However, previous studies showed quantity of cigarettes per day might be associated with worse prognosis in some cancer patients. Also, Agnihotri et al. (2009) evaluated the effect of smoking on salivary antioxidants in subjects with chronic periodontitis, and reported that antioxidants levels were lower in heavy smokers, compared to light smokers and nonsmokers\textsuperscript{15}. In this study we separated smokers to two groups of heavy and light smokers and we matched the year of smoking between two smoker groups. The results of the present study showed the difference in the amount of salivary TAC in light CS compares to heavy CS as well as difference in the alteration of TAC after green tea consumption. It seems that worse prognosis of cancer patients due to quantity of smoking might be due to cumulative stressors and oxidative damage and could be partly compensate with green tea consumption. The higher rates of change in salivary TAC in heavy smokers after GT drinking supports the better effectiveness of GT on heavy CS than NS in the same time period. Ellinger and team (2011) in a systematic review on antioxidant effect of GT consumption concluded that regular consumption of GT may increase plasma antioxidant capacity and may improve the protection against DNA damage in healthy subjects, they concluded beneficial effects by GT consumption seem to be more likely in subjects such as smokers who exposed to increased oxidative challenge\textsuperscript{31}. 

### 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\textsuperscript{20}. 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\textsuperscript{19, 20, 21}. The effect of green tea on cigarette smoke-induced oxidative damage\textsuperscript{2, 4, 5, 8, 18, 22, 23} 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\textsuperscript{24}. 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\textsuperscript{25}. Also, Al-Awaida et al. in 2014 exposed the albino rat model to cigarette smoke, and reported that oxidative stress; inflammation and tissues damage could be prevented by GT supplementation\textsuperscript{22}. In addition, recent studies investigated the role of GT on saliva. Tavakkol et al. (2013) in Iran, evaluated the effects of GT in chemical laboratory workers on salivary antioxidative biomarkers, and reported daily consumption of one cup of GT can reduce several parameters indicative of oxidative stress\textsuperscript{20}. Narotzki et al. (2013) conducted a study in order to elucidate the interaction between GT and its main polyphenol EGCG (Epigallocatechin 3-gallate), and activity of oral peroxidases (OPO). They observed a rise of OPO activity following addition of GT and in a dose dependent manner. They concluded that tea consumers’ oral epithelium might provide an extra protection against the deleterious effects of hydroxyl radicals, produced by not removing hydrogen peroxides in the presence of metal ions\textsuperscript{20}. In an interventional, crossover trial in elderly subjects, Narotzki et al. (2014) reported saliva TAC was improved by 1.5g GT drinking. However, no changes were observed in saliva oral peroxidase enzymes\textsuperscript{27}. Studies reported the difference in salivary TAC in smokers and non-smokers\textsuperscript{12, 26}. Also, the results of a recent study advocated local compensatory mechanism in saliva due to increase in salivary total antioxidant capacity in patients with head and neck squamous cell carcinoma\textsuperscript{25}. In recent study, we evaluated the role of GT on improvement of salivary antioxidant capacity of smokers and results support the effectiveness of green tea drinking in salivary antioxidants enhancement in smokers after three week\textsuperscript{20}. However, previous studies showed quantity of cigarettes per day might be associated with worse prognosis in some cancer patients. Also, Agnihotri et al. (2009) evaluated the effect of smoking on salivary antioxidants in subjects with chronic periodontitis, and reported that antioxidants levels were lower in heavy smokers, compared to light smokers and nonsmokers\textsuperscript{15}. In this study we separated smokers to two groups of heavy and light smokers and we matched the year of smoking between two smoker groups. The results of the present study showed the difference in the amount of salivary TAC in light CS compares to heavy CS as well as difference in the alteration of TAC after green tea consumption. It seems that worse prognosis of cancer patients due to quantity of smoking might be due to cumulative stressors and oxidative damage and could be partly compensate with green tea consumption. The higher rates of change in salivary TAC in heavy smokers after GT drinking supports the better effectiveness of GT on heavy CS than NS in the same time period. Ellinger and team (2011) in a systematic review on antioxidant effect of GT consumption concluded that regular consumption of GT may increase plasma antioxidant capacity and may improve the protection against DNA damage in healthy subjects, they concluded beneficial effects by GT consumption seem to be more likely in subjects such as smokers who exposed to increased oxidative challenge\textsuperscript{31}. 

### 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

### 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±66.2</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>
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.

Acknowledgements:

This work was supported by Shahid Beheshti University of Medical Sciences, Tehran, Iran

Dr. Azimi is in receipt of an Australian Government Research Training Program Scholarship from the University of Western Australia

Conflict of Interests

None Declared

References

How to cite: