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

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

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)[2, 4, 8]. This antioxidative property lead to potential health benefits associated with GT consumption[9] due to the preventive effect in ROS-related diseases such as cancers[10]. 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[11].

Saliva is the first line of defense against encountered cigarette smoke[12]. It has direct contact with tea constituents. Previous studies showed tea catechins could be measured in saliva even after vigorously rinsing the mouth[5, 13]. Moreover, salivary antioxidant capacity is a good measurement for monitoring antioxidant alternation in smokers[14]. In addition, the results of one study suggested salivary antioxidants are different in heavy smokers compared to light smokers and controls[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 agen[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 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[17] 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[15, 17].

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 [10, 18].

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 (tripyridyltriazine). After reaction, samples were read with a spectrophotometer at maximum absorbance in 593 nm[19]. This method has previously been used for measuring salivary TAC[20]. 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.

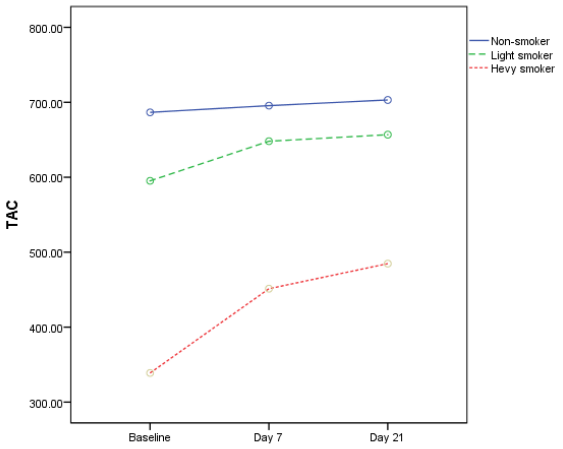

Figure 1- Salivary TAC in light and heavy smokers and non-smokers in study period

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

Group name	Baseline	Day 7	Day 21
NS	686.6±62.2	695.5±57.8	703.1±55.6
Light CS	595.2±46.3	648.1±60.4	656.8±63.2
Heavy CS	338.8±60.0	451.3±60.4	484.8±79.4

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 effects20. 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[9, 20, 21].

The effect of green tea on cigarette smoke-induced oxidative damage[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[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[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[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[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[26]. 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[27].

Studies reported the difference in salivary TAC in smokers and non-smokers [12, 28], 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[29]. 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[30]. 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[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[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

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

1. Kurku H, Kacmaz M, Kisa U, Dogan O, Caglayan O. Acute and chronic impact of smoking on salivary and serum total antioxidant capacity. J Pak Med Assoc. 2015 Feb;65(2):164-9.
2. Chan KH, Ho SP, Yeung SC, So WH, Cho C, Koo MW, et al. Chinese green tea ameliorates lung injury in cigarette smoke-exposed rats. Respir Med. 2009 Nov;103(11):1746-54.
3. Bakhtiari S, Baharvand M, Anbari F, Azimi S, Taheri JB . Effect of vitamin C on salivary superoxide dismutase activity in smokers. Afr J Biotechnol . 2011 June;10(37):7267-70.
4. Misra A, Chattopadhyay R, Banerjee S, Chattopadhyay DJ, Chatterjee IB. Black tea prevents cigarette smoke-induced oxidative damage of proteins in guinea pigs. J Nutr. 2003 Aug;133(8):2622-8.
5. Banerjee S, Maity P, Mukherjee S, Sil AK, Panda K, Chattopadhyay D, et al. Black tea prevents cigarette smoke-induced apoptosis and lung damage. J Inflamm (Lond). 2007 Feb 14;4:3.
6. Huang C-C, Lee W-T, Tsai S-T, Ou C-Y, Lo H-I, Wong T-Y, et al. Tea consumption and risk of head and neck cancer. PLoS One. 2014 May 5;9(5):e96507.
7. Xylinas E, Kluth LA, Rieken M, Lee RK, Elghouayel M, Ficarra V, et al. Impact of smoking status and cumulative exposure on intravesical recurrence of upper tract urothelial carcinoma after radical nephroureterectomy. BJU Int. 2014 Jul;114(1):56-61.
8. Nagaya N, Yamamoto H, Uematsu M, Itoh T, Nakagawa K, Miyazawa T, et al. Green tea reverses endothelial dysfunction in healthy smokers. Heart. 2004 Dec;90(12):1485-6.
9. Hakim IA, Harris RB, Brown S, Chow HS, Wiseman S, Agarwal S, et al. Effect of increased tea consumption on oxidative DNA damage among smokers: a randomized controlled study. J Nutr. 2003 Oct;133(10):3303S-3309S.
10. Erba D, Riso P, Bordoni A, Foti P, Biagi PL, Testolin G. Effectiveness of moderate green tea consumption on antioxidative status and plasma lipid profile in humans. J Nutr Biochem. 2005 Mar;16(3):144-9..
11. Radoï L, Paget-Bailly S, Menvielle G, Cyr D, Schmaus A, Carton M, et al. Tea and coffee consumption and risk of oral cavity cancer: results of a large population-based case-control study, the ICARE study. Cancer Epidemiol. 2013 Jun;37(3):284-9.
12. Baharvand M[1], Maghami AG, Azimi S, Bastani H, Ahmadieh A, Taghibakhsh M. **Comparison of superoxide dismutase activity in saliva of smokers and nonsmokers.** South Med J. 2010 May;103(5):425-7.
13. Yang CS, Lambert JD, Ju J, Lu G, Sang S. Tea and cancer prevention: molecular mechanisms and human relevance. Toxicol Appl Pharmacol. 2007 Nov 1;224(3):265-73.
14. Bakhtiari S, Azimi S, Mehdipour M, Amini S, Elmi Z, Namazi Z. Effect of Cigarette Smoke on Salivary Total Antioxidant Capacity. J Dent Res Dent Clin Dent Prospects. 2015 Fall;9(4):281-4.
15. Agnihotri R, Pandurang P, Kamath SU, Goyal R, Ballal S, Shanbhogue AY, et al. Association of cigarette smoking with superoxide dismutase enzyme levels in subjects with chronic periodontitis. J Periodontol. 2009 Apr;80(4):657-62.
16. Tsao AS, Liu D, Martin J, Tang X-m, Lee JJ, El-Naggar AK, et al. Phase II randomized, placebo-controlled trial of green tea extract in patients with high-risk oral premalignant lesions. Cancer Prev Res (Phila). 2009 Nov;2(11):931-41.
17. Boulos DN, Loffredo CA, El Setouhy M, Abdel-Aziz F, Israel E, Mohamed MK. Nondaily, light daily, and moderate-to-heavy cigarette smokers in a rural area of Egypt: a population-based survey. Nicotine Tob Res. 2009 Feb;11(2):134-8.
18. Wang D, Chen C, Wang Y, Liu J, Lin R. Effect of black tea consumption on blood cholesterol: a meta-analysis of 15 randomized controlled trials. PLoS One. 2014 Sep; 9(9) e107711
19. Benzie IF, Strain J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996 Jul 15;239(1):70-6.
20. Tavakol HS, Akram R, Azam S, Nahid Z. Protective effects of green tea on antioxidative biomarkers in chemical laboratory workers. Toxicol Ind Health. 2015 Sep;31(9):862-7.
21. Lobo V, Patil A, Phatak A, Chandra N.. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010 Jul;4(8):118-26.
22. Al-Awaida W, Akash M, Aburubaiha Z, H Talib W, Shehadeh H. Chinese green tea consumption reduces oxidative stress, inflammation and tissues damage in smoke exposed rats. Iran J Basic Med Sci. 2014 Oct;17(10):740-6.
23. Yuan JM. Green tea and prevention of esophageal and lung cancers. Mol Nutr Food Res. 2011 Jun;55(6):886-904.
24. Schwartz JL, Baker V, Larios E, Chung FL. Molecular and cellular effects of green tea on oral cells of smokers: a pilot study. Mol Nutr Food Res. 2005 Jan;49(1):43-51.
25. Hakim IA, Chow H-HS, Harris RB. Green tea consumption is associated with decreased DNA damage among GSTM1-positive smokers regardless of their hOGG1 genotype. J Nutr. 2008 Aug;138(8):1567S-1571S.
26. Narotzki B, Levy Y, Aizenbud D, Reznick AZ. Green tea and its major polyphenol EGCG increase the activity of oral peroxidases. Adv Exp Med Biol. 2013;756:99-104.
27. Narotzki B, Reznick A, Mitki T, Aizenbud D, Levy Y. Green tea drinking improves erythrocytes and saliva oxidative status in the elderly. Adv Exp Med Biol. 2015;832:25-33..
28. Bakhtiari S, Bigom Taheri J, Bakhshi M, Mortazavi H, Shah Hoseini A, Vahid Dastjerdi E, et al. Effect of vitamin C on salivary total antioxidant capacity in smokers. Iran J Pharm Res. 2012 Fall;11(4):1045-9.
29. Wesołowski P, Zawada K, Wojtowicz A, Strużycka I, Kamiński T. Assessment of salivary total antioxidant capacity in patients with primary untreated head and neck squamous

cell carcinoma with ORAC. J Oral Pathol Med. 2016 Nov;45(10):753-757.
30. Azimi S, Mansouri Z, Bakhtiari S, Tennant M, Kruger E, Rajabibazl M, Daraei A. Does green tea consumption improve the salivary antioxidant status of smokers? Arch Oral Biol. 2017 Jun;78:1-5.
31. Ellinger S, Müller N, Stehle P, Ulrich-Merzenich G. Consumption of green tea or green tea products: is there an evidence for antioxidant effects from controlled interventional studies? Phytomedicine. 2011 Aug 15;18(11):903-15.

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