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Does green tea consumption improve the salivary antioxidant status of smokers?

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ABSTRACT

Objective: Considering the higher rate of oral cancer, and reduction in salivary antioxidants in smokers as indicated in previous studies, antioxidant- containing nutrients such as green tea, seem to be beneficial in counteracting against oxidative stress in this group. This study assessed the salivary total antioxidant alteration in smokers compared to nonsmokers, after short-tem (7 days) and long-term (3 weeks), green tea drinking.

Design: In this experimental study, 20 volunteer moderate-to-heavy male smokers, and 20 matched healthy non-smokers were selected to participate, according to the inclusion criteria. Participants were instructed to drink two cups of green tea per day, by dissolving 2 g of green tea in 150 ml of hot water for each cup. After saliva collection, antioxidant capacity of saliva was measured at baseline, after 7 days, and after 21 days. Statistical evaluation was done by SPSS 21, using paired samplet tests, one-way ANOVA and Bonferroni tests.

Results: At day zero nonsmokers had a higher antioxidant capacity than smokers (686.6 ± 62.22 vs. 338.8 ± 59.9) mM/50 μl, $P < 0.001$. There was also a significant difference between two groups in salivary total antioxidant capacity after one week and three weeks of green tea consumption ($P < 0.001$). However, there was an upward trend in both smokers and non-smokers over the study period (after tea drinking). In addition, a significant difference was found in total antioxidant capacity alteration in smokers compared to non-smokers from baseline to day 21.

Conclusions: Results support the effectiveness of green tea consumption in salivary antioxidants enhancement in smokers, in both the short- and long term.

1. Introduction

Tea prepared from the leaves of Camellia sinensis, is a mixture of a large number of bioactive compounds including catechins, flavonols, lignans, and phenolic acids. Differences in processing of leaves result in different types of tea. Fresh tealeaves steamed immediately after harvesting in processing green tea (GT), result in minimal oxidation of the naturally occurring polyphenols (Hara et al., 2012; Yuan, 2011). Tea polyphenols are strong antioxidants and radical scavengers that are a potential mechanism for the chemopreventive effect against carcinogenesis (Lambert & Elias 2010; Yuan, 2011).

Smoking is a serious global public health problem; cigarette smoke contains numerous compounds that generate reactive oxygen species (ROS), which trigger oxidative damage to DNA and cellular components, thereby contributing to carcinogenesis (Arazi, Simaei, & Taati, 2015; Kurku, Kacmaz, Kisa, Dogan, & Caglayan, 2015). Due to the popularity of cigarette smoking and tea drinking, several experimental studies have explored the possible inhibitory effects of tea on cancer formation induced by cigarette smoking (Kurku et al., 2015). In human experiments, Schwartz, Baker, Larios, and Chung (2005) and Hakim et al. (2003) have shown that GT treatment lead to reducing DNA damage in smokers. These findings demonstrated the antioxidative property of GT in

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anticarcinogenesis (Yuan, 2011). Moreover, two small trials in patients with premalignant oral lesions demonstrated that the administration of tea resulted in a reduced risk of oral cancer (Dash et al., 2012; Li, Sun, Han, & Chen, 1999; Tsao et al., 2009) however, it is not known whether tea has a role in the primary prevention of oral cancer, or the effect of tea on oral cells might be different in smokers than non-smokers (Dash et al., 2012). In an intervention study ingestion and topical application of green tea significantly decreased the size of oral lesions in comparison to the untreated controls (Li et al., 1999).

Saliva is the first biological medium encountered during inhalation of cigarette smoke and first line of defense against oxidative stress. The antioxidant system of saliva has protective effects against microorganisms, toxins and oxidants, so may play a role in the anti-carcinogenic effect (Baharvand et al., 2010). Based on the previous studies, oxidant-antioxidant balance of saliva is degraded in favour of oxidants in smokers, and this per se, contribute to oral hygiene deterioration and oral cancer development in smokers (Bakhtiari et al., 2012, 2015).

Carcinogenesis is a multistage process (Lee & Choi 2011). Constant and direct attacks of smoking on the oral epithelial cells gradually accumulate and lead to stepwise malignant transformation (Sedighe, Maryam, Fahimeh, Somayyeh, & Bigom, 2013). Thus it has many phases for prevention and intervention. Green tea is an ideal agent for a chemoprevention study because it has a low cost and toxicity and is readily available. Considering the inconclusive outcome about the protective effects of green tea consumption against the development of oral cancers in humans (Lee & Choi 2011) and given the high performance of saliva in demonstrating the negative impact of smoking both locally and systematically (Kurku et al., 2015), the aim of the present study was to evaluate the effect of short-term (after 7 days) and long-term (after 3 weeks) green tea consumption on total salivary antioxidant capacity (TAC) in smokers, to determine the rate of change in TAC, with the daily intake of green tea.

2. Methods and materials

2.1. Ethics

The study was conducted according to the principles of the "Declaration of Helsinki"; and approved by the Ethics Committee of the local medical University (IR.SBMU.RIDS.REC.1394.76). All participants signed an informed consent document prior to the study.

2.2. Participants

In this experimental study, 40 healthy male moderate- to-heavy cigarette smokers (CS) and non-smokers (NCS) were studied. Sample size was determined with power and sample size calculation software, version 3.0.43 (power 80%, P value <0.05), considering 20% potential loss of participants. Twenty moderate-to- heavy CS; (defined as those who ever smoked 100 cigarettes, smoked daily in the past 30 days, and smoked more than 10 cigarettes/day) (Boulos et al., 2009); and 20 matched control NCS; (who were self-reported of never smoked); were selected through a convenience sampling method. The participants have been recruited from attendees to the oral medicine department, Shahid Beheshti University of Medical Sciences, Tehran, Iran for routine dental checkups, and who met the inclusion criteria, during a 2-month period (starting December 2015). Adult healthy Persian males were included (considering the case definition), who: had at least 20 teeth; without 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. Also, participants were examined for clearance from oral lesions before inclusion in the study.

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.

2.3. Experimental protocol

All participants were asked to consume a total of two cups of green tea per day between breakfast and lunch and between lunch and dinner for the period of three weeks. Every cup was prepared by infusing 2 g of GT in 150 ml of hot water (80 °C) for 3 min. To reduce variation in consumption of GT, all packages of green tea from same brand (Golestan brand) with the same production date and batch types were purchased. We supplied GT in small tea bags after careful weighing with a digital scale. Also, we provided the same glasses to each participant for equalizing the amount of heated water. They could add sugar to the tea, but addition of milk was not allowed. The bags of tea were labeled with each subject's number, and they were asked to stick their labels in a daily diary as a compliance check. The subjects 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 in order to match the amount of antioxidants among participants as much as possible (Erba et al., 2005; Princen et al., 1998).

2.4. Saliva collection

Whole unstimulated saliva was collected at baseline, after one week and after 3 weeks of tea consumption. After rinsing the mouth with 15 ml of distilled water, subjects had to spit almost 2 ml of their saliva into falcon tubes in an upright position, between 9 and 12 o'clock in the morning. They had been requested to avoid eating, drinking or smoking at least 1 h before the saliva collection. The samples were immediately centrifuged at 3000 (rpm) at 4 °C for 15 min; then was stored at −70 °C until analysis. The trained senior students and the technician were blind about the participants.

2.5. Total antioxidant capacity assay (TAC)

TAC was measured by the ferric reducing ability of plasma (FRAP) method. This method is a valid method for measuring antioxidant capacity, 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 the spectrophotometer at maximum absorbance in 593 nm (Benzie & Strain 1996). This method has previously been used for measuring salivary TAC (Tavakol, Akram, Azam, & Nahid, 2013). In this study, all samples were measured at the same time with one solution, and results were read with one calibrated spectrophotometer (SHIMADZU). Also, one experienced technician (who was blind about cases) completed all procedures, under supervision of a clinical biochemistry specialist.

2.6. Statistical analysis

Results are expressed as mean ± standard deviation. The differences between groups were assessed by paired samplet tests, One-way ANOVA and Bonferroni tests. Statistical significance was set at 95% ($P < 0.05$). SPSS software (Version 21) was used for statistical analyses.