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EFFECT OF GREEN TEA (CAMELLIA SINENSIS) ON THE LEVEL OF THE SALIVARY TOTAL ANTIOXIDANT CAPACITY (TAC) IN A SAMPLE OF PERIODONTAL PATIENTS

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ABSTRACT

This study was designed to investigate whether the green tea intake will affect the salivary antioxidant level in a sample of periodontal patients. The study was carried out on a sample of male periodontal patients with age ranging from 25-45 years. The subjects were divided to 3 groups; *Group A:* which include Thirty subjects with no clinical and/or radiographic, *Group B*: Thirty subjects diagnosed of periodontitis who had no history of green tea intake or any antioxidants for at least 3 months and *Group C:* Thirty subjects of group B after green tea intake three times daily. The level of salivary antioxidants was measured for both study and control groups via estimation of Total salivary antioxidant capacity (TAC). The results of this study showed significant decrease in the level total antioxidant capacity (TAC) in group B groups compared to that of group A .On the other hand,significant increase in the level total antioxidant capacity (TAC) of group C compared to both group B

According to this study, we could conclude that green tea consumption may prove to be a promising adjunctive prophylactic and therapeutic modality in reducing oxidative damage in saliva of periodontal patient

INTRODUCTION

Saliva is the mirror of oral health. It is rich in antioxidants which constitutes another defence mechanism and appears to be of paramount importance [1,2]

Free radical such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated by various endogenous protective systems in our body and /or exposure to different physiochemical conditions or pathological states

Imbalance between Reactive Oxygen Species (ROS) production and antioxidant defense inside human organism result in Oxidative stress [3,4] Oxidative stress is implicated in the pathogenesis of many oral diseases as dental caries and periodontal diseases [5-7] Periodontal disease is a chronic disease that is more prevalent in adults. It has been shown

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that periodontal disease is associated with reduced levels of antioxidants enzymes[and excess release of reactive oxygen species (ROS). The removal of ROS by antioxidants is essential for healthy periodontium. [8,9]

Antioxidants such as polyphenols can neutralize the free radicals and may reduce or even help prevent some of the damages. Polyphenols are contained in some herbal compounds as green tea. Green tea contains many polyphenolic components known as catechins such as epigallocatechin gallate (EGCG) and epigallocatechin, which possess higher antioxidant activity than Vitamin C and E. Their antioxidant action involves scavenging of ROS or chelation of transition metals while indirectly, they upregulate Phase II antioxidant enzymes [10]

The total capacity of all antioxidants present in our body to neutralize the ROS, is known as total antioxidant capacity (TAC). TAC is one of the salivary diagnostic markers, which contains two groups of enzymatic and non-enzymatic antioxidants and it is the first line of defense against oxidative stress[2,8,11-13]

The aim of this study was to evaluate only TAC in saliva as an indicator of oxidative damage. Furthermore, deduce the effect of green tea intake on the level of the salivary total antioxidant capacity (TAC) in periodontal diseases.

Selection Criteria

A total of sixty male subjects of age range (25- 45) were enrolled in this study Subjects are apparently healthy and not suffering from any systemic diseases as evaluated by Cornell Index[14] and by the modified Cornell medical index.[15] A written consent was signed by all participants included in the study

Exclusion Criteria

Subjects were excluded if the following conditions

- History of diabetes, hepatic diseases and/ or any systemic diseases
- History of salivary gland surgery and /or history of smoking or alcohol intake.
- Chronic use of drugs that affect periodontal conditions history of alcohol intake and /or subjects who received professional hygiene or periodontal therapies in the 6 months preceding the screening
- Regular consumption of more than one cup of green tea or 3 cups of black tea or coffee daily
- Regular users of mouthwash, medications or vitamin supplements within the past 3 months,

Periodontal analysis

All subjects were clinically examined in a standardized manner *Plaque index(PI), Gingival index(GI), Probing depth (PD) and Clinical Attachment Level (CAL) measurements were recorded*. [16-19]

Examination instruments were adequately packed in sterilization bags and submitted to sterilization at National Center of Radiation Research and Technology (NCRRT) using cobalt -60 gamma source (GB50 Type B, Canada) at a dose of 25KGy for 6 hours under room temperature

Based on the clinical periodontal parameters, smoking status and selection criteria, subjects were divided into the three following groups:

Group A **(Control group):** Thirty subjects with no clinical and/or radiographic manifestations of periodontal disease

Group B**:** Thirty subjects diagnosed of periodontitis (a minimum of six periodontal pockets **≥**5 mm or the loss of attachment of ≥3 mm) who had no history of green tea intake or any antioxidants for at least 3 months

Group C**:** Thirty subjects of group B after intake and of green tea three times daily for 45 days

The green tea used in the study was (Ahmed green tea leaves sachets) Each individual sachet contained 1.6 g of ground green tea leaves. The solution of oral

mouthwash was prepared by infusion of the sachet in 200 mL of recently boiled filtered water, where it was allowed to steep for 5 minutes. The sachet was then removed, and the solution was allowed to cool naturally then used in taken orally.

Estimation of Total Phenol Content (TPC) of green tea

The total phenol content (TPC) of cold and hot extract of green tea leaves extracts were determined by Folin Ciocalteu method Briefly, 200 µl of diluted extract was added to a test tube and then mixed with 1000 µl of Folin–Ciocalteu reagent (1:10). Thirty seconds later and just prior to 8 min, 800 µl of Na2CO3 (7.5%) was added. The reaction mixture was incubated at 24 °C for 1 h before absorbance (at 765 nm) of mixtures was recorded against blank. Total phenolics of extracts were calculated from standard gallic acid solutions (0– 0.1 mg/ml), and expressed as mg gallic acid equivalents (GAE) per g extract.

The amount of TPC was higher in hot water extract 123 µg CAE/mL. compared to cold extract 88 µg CAE/mL.

Measurement of total antioxidant capacity of saliva

Total antioxidant capacity (TAC) was measured by spectrophotometric method [20]

Principle: A standardized solution of Fe-EDTA [Iron- Ethylene diaminetetra acetic acid] complex reacts with hydrogen peroxide by a Fenton-type reaction, it leads to the formation of hydroxyl radicals (OH). These ROS degrade benzoate, resulting in the release of thio barbituric acid reactive substances (TBARS). Antioxidants from the added sample of human fluid (saliva) cause suppression of the production of TBARS. This reaction can be measured spectrophotometrically and the inhibition of color is development defined as the antioxidant activity

Statistical analysis

Data presented as mean and standard deviation (SD). Students'-test was applied for detecting the significance of difference between groups. $P \leq 0.05$ was considered to be statistically significant. Statistical analysis was performed using SPSS software (PASW, Windows version 18.0).

RESULTS

The total antioxidant activity was reduced significantly in group B compared to group A control group on the other hand, Significant increase of The total antioxidant activity in group C compared to group B and group A (control group) was recorded.

TABLE (1) Shows total antioxidant activity between compared groups

GROUP / Parameter	GROUP A (control)	GROUP B	GROUP C
Saliva TAC level (mmol/L)	1.038 ± 0.88	0.361± 0.43***	0.816 ±0.87**

Data are presented as mean± SD, n=30 * Significant difference ** High significant difference ***Very high significant difference

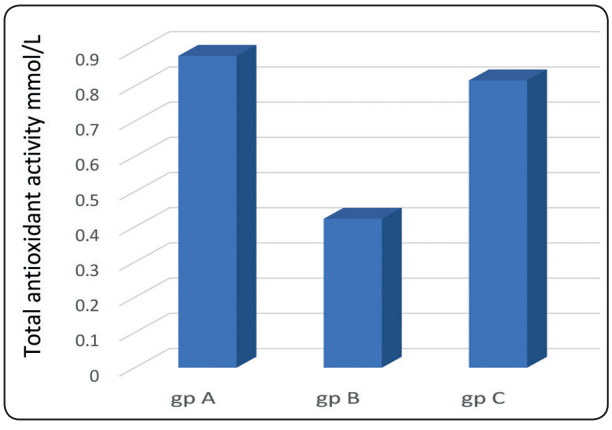

Fig. (1) Histogram showing mean Laboratory parameter values for the difference tested group

DISCUSSION

The present study was conducted on male subjects of age range (25-45) years.Tight matching for gender, age and exclusion of smoking was considered so as to reduce the potential confounding factors.(**Shern *et al* 1993, Percivals *et al* 1994), Fenol *et al* 2004, Celecová *et al* 2013 and Motamayel *et al* 2013**).

Saliva samples were collected from all subjects in both the study groups and the control group since it presents many advantages as a diagnostic tool. Saliva is easy to collect by a non-invasive technique, no special equipment is needed, and collection is associated with fewer compliance problems compared with blood collection. Furthermore, analysis of saliva may provide a cost-effective approach for the screening of large populatirbidimetryons**. (Kauffman and Lamster 2002, Miricescu *et al* 2011) .**

In our study, total antioxidant capacity (TAC) was chosen to be the parameter to determine the antioxidant defence owing to the fact that, it includes all salivary antioxidants; it presents clinical significance in the evaluation of the antioxidant status of saliva under normal and pathological situations. Moreover, it was found to be the most relevant parameter for assessing the defence capabilities. **(Miricescu *et al* 2011and Pendyala *et al* 2013)**

The unstimulated saliva was preferred to stimulated saliva as it was claimed that the total antioxidant capacity is higher in unstimulated saliva **(Miricescu *et al* 2011and Pendyala *et al* 2013)**

The present study resulted in significant decrease in the total antioxidant capacity (TAC)between group B (periodontal patients) compared with group A (control). This was constituent with These findings are in accord with (**Chapple *et al* 1997, Diab-Ladki *et al* 2003, Sculley and Langley-Evans 2003, Brock *et al* 2004, Trivedi *et al* 2015 and Chang *et al* 2018)** who reported a significant decrease in total antioxidant activity of saliva in patients with periodontitis when compared to healthy individuals.

The recorded results could be explained by the fact that chronic periodontal disease is associated with hyper-reactive peripheral neutrophils. This result in over production of the production of ROS in response to Fcγ-receptor stimulation

Regarding the effect of green tea on the level of total salivary antioxidant (TAC). The present study, found that there was a significant increase in the level of on the level of total salivary antioxidant (TAC) in periodontal patients after green tea intake (group C) in comparison of patients who reported no history of green tea or any antioxidant (group B). This was in constituent with(**Tavakkol *et al* 2013, Narotzki *et al* 2014**, **Azimi *et al* 2018 and Rafieian *et al* 2019)** who supported the role of green tea drinking in Increase of TAS level and reducing oxidative. Moreover, (**Chandra *et al* 2013, Reddy *et al* 2015, Szulińska *et al* 2017 and Tripathi *et al* 2019**) who found a direct positive correlation between periodontal health and adjunctive antioxidant supplementation

This could be attributed to green tea antioxidant action which involves scavenging of ROS or chelation of transition metals while indirectly, they upregulate Phase II antioxidant enzymes. Consequently, it re0duces oxidative stress, improves the antioxidant status, and decreases markers of inflammation[10,46]

CONCLUSION

Based on the results from the present study, it was proved that:

- Estimation of the TAC may serve as an indicator to assess the prognosis of periodontitis
- Green tea consumption may prove to be a promising adjunctive prophylactic and therapeutic modality in reducing oxidative damage in saliva of periodontal patients

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