

Biochemical Evaluation of the Effect of Green Tea on Nitric Oxide Radical in Patients with Chronic Periodontitis

Jahangirnezhad, M.* Ghaffari, S.M.** Khosravi Farsani, L.*** Jalali, F.***

*Associate Professor. Department of Periodontology, Faculty of Dentistry, Ahwaz Jundishapour University of Medical Sciences, Ahwaz, Iran.

**Associate Professor, Department of Biochemistry, Faculty of Medicine, Ahwaz Jundishapour University of Medical Sciences, Ahwaz, Iran.

***Doctor of Dental Medicine, Ahwaz Jundishapour University of Medical Sciences, Ahwaz, Iran.

ABSTRACT

Statement of the Problem: Periodontal disease is a periodontal tissue inflammation caused by gram-negative and other bacteria. The release of various cytokins and mediators, including nitric oxide, in the inflammation area usually has detrimental effects on the periodontium, potentiating the inflammatory process. In order to prevent this side effect, investigators have used anti-oxidants such as green tea or nitric oxide synthase inhibitors as a remedy.

Purpose: In this study the effect of green tea on salivary nitric oxide radical was investigated in patients with chronic periodontitis.

Materials and Methods: Two groups of patients (n=10), suffering from chronic periodontitis as control and case groups, were evaluated. The case group used green tea for a period of four weeks. The control group received no intervention. At the end of this period the salivary total nitrite concentration as an indicator of nitric oxide was measured by ELISA technique based on Griess reaction. The results were compared statistically by paired-t-test at $P < 0.05$.

Results: Green tea reduced the salivary concentrations of nitric oxide significantly ($P = 0.000$) in the case group in comparison with the control group which received no treatment.

Conclusion: The results indicated that green tea may be considered a useful herb for treatment of chronic periodontitis.

Keywords: Green tea, Nitric oxide, Periodontitis.

INTRODUCTION

Chronic periodontal inflammation is known as periodontitis which is mainly triggered and accentuated by bacterial causes.⁽¹⁾ This immune process and any other inflammatory cascade are affected by humoral and cellular immune elements, among which nitric oxide (NO) can be mentioned.⁽²⁾ This biochemical substance which is synthesized from L. arginine by inducible nitric oxide synthase (iNOS) plays a protective role in infectious inflammatory diseases. The production rate of this enzyme (iNOS) and hence the release of nitric oxide by immune-competent cells, such as macrophages, is accelerated during infection.⁽³⁻⁵⁾ A link has been reported between this excess

Corresponding author: M. Jahangirnezhad, Address: Department of Periodontics, Ahwaz Jundishapour University of Medical Sciences, Ahwaz, Iran. Tel: +98 611 320 51 69 Fax: +98 611 336 43 66 Email: drmahjahan@yahoo.com

production of nitric oxide and periodontal disease and tissue damage seen in this pathology;^(3,6) in addition, the usefulness of salivary nitric oxide as a biochemical marker of periodontal disease has been proposed.^(7,8) Based on the reports proposing an important role for nitric oxide in etiopathogenesis of periodontal diseases some investigators employed iNOS inhibitors and anti-oxidants in order to treat periodontitis. Green tea is one of the anti-oxidants used.^(9,10)

As there are conflicting reports in relation to salivary concentrations of NO in periodontitis⁽¹¹⁾ this study was undertaken in order to evaluate use of green tea as a remedy in periodontitis.

MATERIALS AND METHODS

A group of twenty patients with severe chronic periodontitis, confirmed by clinical parameters obtained through observations, participated in this study. The protocol of

the investigation was approved by the Ethics Committee of the university.

Inclusion criteria

- 1-Clinical attachment loss of ≥ 5 mm, measured using Williams periodontal probe
- 2-Bleeding on probing

Exclusion criteria

- 1-Subjects who required antibiotic or anti-inflammatory drug therapy
- 2-History of any systemic diseases
- 3-Subjects who were pregnant and pre-eclamptic
- 4-Subjects with a history of smoking and tobacco use
- 5-Subjects with vitamin supplements
- 6-Subjects who regularly used mouthwashes

The patients were randomly divided into two groups of ten (n=10). One group was considered as the control (no use of green tea) and the other used green tea (1.75 g of tea leaves in 200 mL of water) for a period of four weeks and was considered a the case group.⁽¹²⁾ At the beginning of the experiment and before any intervention a sample of 10 mL of unstimulated salivary samples were collected, centrifuged and the supernatant was frozen at -20°C and stored as base-line samples. After four weeks of treatment of the case group with green tea, again the same salivary samples were collected from the control and case groups.

The NO was measured in the above salivary samples employing Elisa Kit. Based on Griess reaction, the results were analyzed and compared using paired-t-test. Statistical significance was defined at $P < 0.05$.

RESULTS

Total salivary nitrite was measured as nitric oxide index by Griess reaction. There were no significant differences in salivary nitric oxide concentrations between the two groups (A&C) at the beginning of the experiment ($P=0.0753$).

Significant differences were observed in the salivary nitric oxide concentrations between the control group at the beginning of the experiment (C) and the case and control groups after intervention (B) ($P=0.001$).

NO salivary concentrations were significantly different in the case group (A) before intervention and the control group (D) at the end of the the experiment ($P=0.000$).

A significant difference was observed between NO salivary concentrations in the control group at the beginning (C) and at the end (D) of the experiment ($P=0.000$).

Significant differences ($P=0.000$) were observed in NO salivary concentrations in the case group before (A) and after (B) the intervention (Table 1).

Table 1: Comparison of NO salivary concentrations in the case and control groups at the beginning and at the end of the experiment

		N	Mean	Std. Deviation	Std. Error Mean
Nitrite	A	9	4.9489	1.14602	0.38201
	B	9	2.4522	1.14808	0.38269
	C	8	5.1575	1.53026	0.54103
	D	8	9.8338	1.80885	0.63953

DISCUSSION

Immunological response to bacterial infection in the periodontium results in accumulation of various kinds of biochemical mediators in addition to immune-competent cells in the inflamed periodontium.^(2,13) This defense reaction is accompanied with some clinical and biochemical signs in the affected periodontal tissue and biological fluids surrounding the area, including saliva, which provide the basis for the diagnosis and classification of periodontal diseases.^(14,15)

Cytokins produced as a result of bacterial invasion of periodontium trigger resident and/or immigrant cells for the expression of iNOS which in turn produces high levels of nitric oxide and its highly reactive metabolite known as peroxynitrite in the inflammation site.^(13,16) As various reports have proposed a role for nitric oxide in the progression of periodontal disease⁽¹³⁾ some investigators suggested the selective inhibition of iNOS and scavenging of peroxynitrite^(9,17,18) or neutralization of reactive species by anti-oxidant substances⁽¹⁰⁾ as a promising novel approach for the treatment of periodontitis.

In this project the aim was focused on neutralizing nitric oxide reactive metabolite by green tea as an anti-oxidant product, thereby lowering the speed of progression of periodontitis or treating it. The results of this study documented the lowering effect of green tea on salivary nitric oxide concentration known as biochemical marker of periodontitis in comparison with the control group (P=0.000). Proper clinical

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effects of green tea on the affected periodontium were established in this department in a similar group of patients using the same dosage of green tea as used in this work.⁽¹⁰⁾

This effect of green tea on NO salivary concentrations has also been reported by others.^(10,19)

In the study by Maruyama and Tomofuji the effects of a dentifrice containing green tea catechins on gingival oxidative stress and periodontal inflammation was evaluated using a rat model and it was concluded that incorporation of green tea catechins into a dentifrice may contribute to prevention of periodontal inflammation by decreasing gingival oxidative stress and expression of pro-inflammatory cytokines.⁽²⁰⁾

In addition, the result clearly showed that the uninterrupted periodontitis process will result in accumulation of excess amounts of nitric oxide in saliva (Table 1) (P=0.000), consistent with another report.⁽²¹⁾

CONCLUSION

The results showed that green tea may be considered a suitable remedy for periodontitis. However, as the number of the participant patients in this project was limited, it is suggested that this work be followed by exercising the same plan on a larger group of patients.

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