Immunological response of gingival tissue injected by green tea

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ABSTRACT

Background: Green tea has many beneficial effects on the body. The recent era is all about herbal treatment of various diseases and green tea is one of the best tonics for healthy well beings. This study examined the immunological response of sulcular injection of green tea on gingival health using a rabbit model.

Methods: Twenty male rabbit weighted 1-1.5 kg of the same species. Blood samples were taken for each normal rabbits before sulcular injection with 5% green tea solution, blood drown after three days of the injection for immunological assessment by measuring of IL-1β, TNF-α and INF-γ.

Results: Study showed there was a highly significant decrease in mean concentration of IL-1β, TNF-α after injection with 5% green tea solution(P< 0.01), while a significant elevation was observed in mean concentration of INF-γ after injected with the same solution(P<0.05).

Conclusion: Green tea sulcular injection had beneficial effect on the periodontal health condition, thus green tea can be used safely and successfully in oral field.

Key words: Green tea, cytokines, sulcular injection, gingival health. (J Bagh Coll Dentistry 2012; 24(4):95-99).

INTRODUCTION

Tea is consumed worldwide, although in greatly different amounts; it is generally accepted that, next to water, tea is the most consumed beverage in the world [1]. Green tea polyphenols are responsible for its antioxidant activity either directly by scavenging of reactive oxygen and nitrogen species and chelating redox-active transition of metal ions like iron and copper or indirectly by inhibition of pro oxidant enzymes, redox sensitive transcription factors, and induction of antioxidant enzymes [2]. Green tea catechins were reported to be effective in preventing gingival and periodontal inflammation. Epigallocatechin-3-gallate (EGCG) inhibits the m-RNA expression of COX-2, MMP-1 (matrix metalloproteinase-1), IL-1, 6 and 8 by cultured cells. Effective concentration to achieve these effects was ≥1 μg/ml. EGCG inhibit the activation of NF-κB (Nuclear Factor–kappa B), which is one of the key positive regulators of COX- 2 expression [3]. EGCG inhibits IL-1β induced cartilage proteoglycan degradation and expression of MMP-1 and MMP-13 (matrix metalloproteinase-13) (in human chondrocytes at micromolar concentration. Complete inhibition of MMP-1 and MMP-13 at a concentration of 100 μg EGCG was observed. This concentration can be achieved only by local administration and not by oral consumption. MMP-13 is more sensitive to the inhibitory effect even at lower conc.

This inhibitory effect is by inhibition of IL-1β induced expression of m-RNAs signifying that the effect is at transcriptional level [4], so EGCG may inhibit the activities of MMPs involved in the degradation of native collagen and this may have suppressive effects on the cartilage degradation in arthritic joints. Green tea polyphenol (GTP) supplementation mitigated deterioration of bone micro-architecture and improved bone integrity in rats with chronic inflammation by suppressing bone erosion and modulating cancellous and end cortical bone compartments, resulting in a larger net bone volume. Such a protective role of GTP may be due to a suppression of TNF-alpha [5]. The aim of this study was to evaluate of certain immunological response through estimation of parameters like IL-1*B*, INF and TNF-α after intrsulcular injection of green tea into the periodontium. In this study, we examined the immunological response of intrasulcular injection of green tea on gingival health using a rabbit model.

MATERIALS AND METHODS

Sample Collection and study design

This study was carried out at the Hawler Medical University, College of Dentistry, Department of Periodontology, Erbil city during the period from 1st Jan 2012 up to 30th May 2012. Blood samples were collected for each normal rabbits before injection with 5% green tea solution. By Cardiocentesis, blood samples were collected under subcutaneous general anesthesia after an overnight fasting (12 – 14 hours). About 5 ml of blood was collected and dispensed into vacutainer plain tubes. After centrifugation at 3000r.p.m. for five minutes, the serum was stored at – 80 °C. After one week, rabbits injected with

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50 L/Kg [6] sulcular injection of 5% green tea solution to the lower right central incisor. Needle was inserted to a depth of 5mm, the depth of penetration was measured by adjusting the sleeve to stop 5mm from the tip of the needle, and bloods drown after three days of injection with the same solution.

Laboratory animals

Twenty male rabbits of the same species and weight (1-1.5 kg) were left to acclimatization for seven days before starting the experiments, to maintain their standard diet and environmental condition were equal among all animals. Rabbits housed in an air –conditioned room (23-25°C) with a 12-h light- dark cycle. They had free access to water and standard food during the experimental period, tags with different numbers were fixed on the rabbit's ear to mark them.

5% green tea preparation

Green tea was purchased from supermarket, dry Chinese green tea –Temple of Heaven Gun power. Five grams of the selected green tea leafs, steeped for 1.5- 2 minutes in 100 ml of distilled water. The coolest brewing temperature was 160F/69°C.The mixture was purified to obtained the 5% concentration solution of green tea [7].

Immunological assay

Serum IL-1β, TNF-α, INF-γ were measured by using an immunometric kits (KOMA BIOTECHINC, USA). The reader was microplate ELISA reader, DNM-960A, Germany. Quantification of IL-1β, TNF-α, INF-γ were determined by use of enzyme –linked immunosorbent assay kits. Multiple cytokine expression concentrations were simultaneously determined according to the manufacture's instruction.

This study examined the immunological response of sulcular injection of green tea on gingival health using a rabbit model.

Statistical analysis

Data were evaluated statistically, analyzed and organized in tables and graphs. Computer program software SPSS (Statistical Package for Social Sciences); version 17 was used to analyzed the data. Quantitative variables were compared using the Student's t-test and expressed as (Mean ±S.E.M). Results were considered significant, if the P value less than 0.05.

RESULTS

Mean concentration of IL-1 β (pg/ml) in sera rabbit study groups

Table 1 and Figure 1 showed that the mean concentration of IL-1β was higher (41.91pg/ml) in sera of rabbit group (B) when compared with its mean concentration (36.85 pg/ml) in sera of rabbit group (A) after injection with 5% green tea

solution with highly significant difference (P<0.01).

Mean concentration of TNF-α (pg/ml) in sera of study groups

Table 2 and Figure 2 showed that the mean concentration of TNF-α was higher (23.02 pg/ml) in sera of group (B) when compared with its mean concentration (10.71 pg/ml) in sera of group (A) after injection with 5% green tea solution with highly significant difference (P<0.01).

Mean concentration of INF –γ (IU/ml) in sera of study groups

Table 3 and Figure 3 represented that the mean concentration of INF –γ was higher (23.25 IU /ml) in sera of group (B) when compared with its mean concentration (20.11 IU /ml) in sera of group (A) after injection with 5% green tea solution with significant difference (P<0.05).

DISCUSSION

Estimation of serum IL-1β:

This study found that the mean concentration of IL-1β was highly significant decrease in rabbit sera after injection with 5%green tea solution when compared with its mean concentration in rabbit sera before injection (P<0.01). Recent studies reported that IL-1β was a potent inducer of reactive oxygen species (ROS) via enhanced expression of enzyme, inducible nitric oxide synthase (iNOS) [8].

A study done by Singh and colleagues showed that the green tea poly phenol inhibited the IL-1β induced (iNOS) expression and activity [9].

A study which gives support to the above results demonstrated that the green tea poly phenol was found to be effective in reducing IL-1β -induced inflammatory cytokine from condrocyte [10]. Another study found that the alveolar bone resorption and IL-1β expression induced by lipopolysacharide in gingival tissue were significantly decreased by injection or oral administration of green tea catechins (GTC). GTC inhibits nuclear translocation of NF-kappaβ activated by LPS [11].

In contrast to our finding, the results obtained from a study revealed that ECG improved the quality of scarring by inducing iNOS [12].

Estimation of serum TNF-α

This study found that the mean concentration of TNF-α was highly significant decrease in rabbit sera after injection with 5%green tea solution when compared with its mean concentration before injection (P<0.01). The local response to tissue injury involved the production of cytokines, thus increased plasma level of TNF-α at the site of inflammation [13].

A study done by Sutherland and colleagues showed that the catechins' anti-inflammatory effects, in part, due to their scavenging of NO and reduction of NO synthase (NOS) activity [14].

Another study done by Lehnert and colleagues mentioned that the radical scavenging plant polyphenol blunted TNF-α production after hemorrhage, indicating that ROS/RNS contribute to cytokine formation and release [15].

A study which give support to the above results demonstrated that the ROs - scavenging activity of green tea catechins inhibit NF-kappa B activation leading to inhibition of expression of the pro inflammatory and survival genes [16].

Estimation of serum INF –γ

The results of this study showed a significant decrease level of mean concentration of INF–γ in rabbit sera after injection with 5% green tea solution when compared with its mean concentration before injection (P<0.05).

A study done by Tedeschi and colleagues found that drinking green tea exerts a specific inhibitory action of INF–γ [17].

A study which gives support to the above results demonstrated that the reduced collagen induced arthritis incidence and severity was reflected in marked inhibition of the inflammatory mediator INF–γ in arthritis joint of green tea –fed mice [18].

The present study suggests that there is an association between the intake of green tea and periodontal health condition. One can concluded that the anti-inflammatory effects on the low grade inflammation could be enhanced by sulcular injection of green tea by decreasing of IL-1β, TNF-α, INF-γ. Green tea sulcular injection may be expected to have a more beneficial effect on the periodontal condition.

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Table 1: Mean concentration of IL-1β (pg/ml) in sera of rabbits before and after injection with 5% green tea solution

Study Group	No.	Range of Concentration of serum IL-1β (pg/ml)	Mean Conc. of serum IL-1β (pg/ml)	SD	C.S by t-test
(A) After injection	20	31 – 41.5	36.85	2.66	P=0.001 C.S. = H.S
(B) Before injection	20	32.47– 51.02	41.91	6.23	
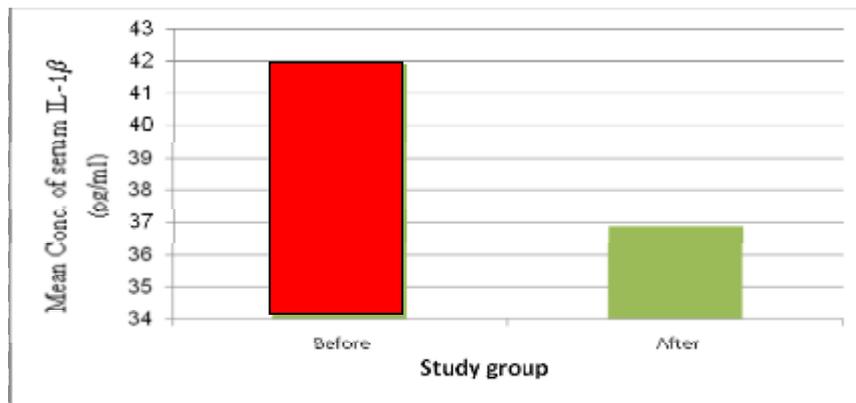
Figure 1: Mean concentration of IL-1β (pg/ml) in sera of rabbits before and after injection with 5% green tea solution.

Table 2: Mean concentration of TNF-α (pg/ml) in sera of rabbits before and after injection with 5% green tea solution

Study groups	No.	Range of Concentration of serum TNF-α (pg/ml)	Mean Conc. of serum TNF-α (pg/ml)	SD	C.S by t-test
(A) after injection	20	9.93 – 12.10	10.71	0.54	P=0.000
(B) before injection	20	15.7 – 48.8	23.02	6.94	C.S. =H.S
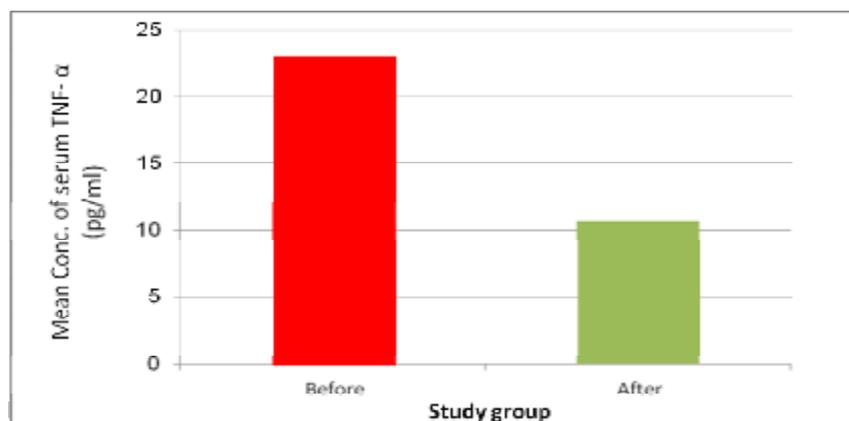
Figure 2: Mean concentration of TNF-α (pg/ml) in sera of rabbits before and after injection with 5% green tea solution

Table 3: Mean concentration of INF-γ (IU/ml) in sera of rabbits before and after injection with 5% green tea solution

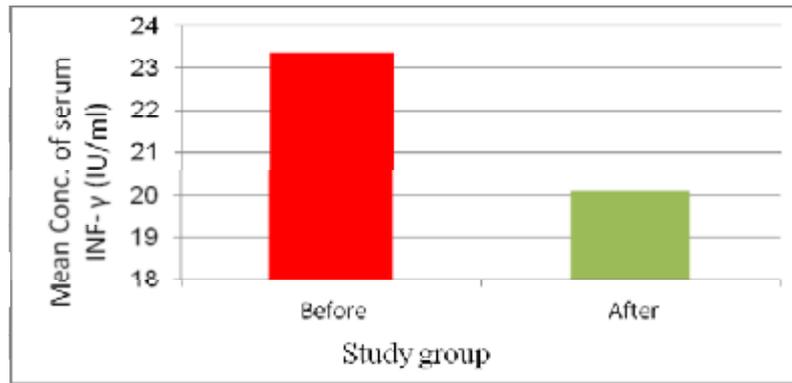| Study groups	No.	Range of Concentration of serum INF-γ (IU/ml)	Mean Conc. of serum INF-γ (IU/ml)	SD	C.S by t-test
(A) after injection	20	11.00 – 24.90	20.11	3.56	P=0.026
(B) before injection	20	20.50 – 33.5	23.25	2.84	C.S.=S

Figure 3: Mean concentration of INF-γ (IU/ml) in sera of rabbits before and after injection with 5% green tea solution