

# The antioxidant effect of sulcular injection of green tea

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## ABSTRACT

**Background:** Green tea is a popular nutraceutical as an antioxidant. The association between tea consumption, especially green tea, and human health has long been appreciated.

**The aim of this study** was to examine the antioxidant effect of sulcular injection of green tea to evaluate its use in periodontal treatment.

**Methods:** Fifty –five male rabbit weighted 1-1.5 kg of the same species divided into three groups. The first group, group A, (test group) received 50µL/Kg of green tea dissolved in distilled water, the 2<sup>nd</sup> group, group B, received distilled water. The last group, group C, (control group) received no injection. Blood samples were taken at a time interval of (1, 3, 42, 72,168) hours for biochemical analysis of vitamin C and Malondialdehyde (MDA).

**Results:** Study showed there was a highly significant increase in mean concentration of serum vitamin C three hours after sulcular injection with 5%green tea extract (P<0.01),while a significant decrease in mean concentration of serum MDA after injection at the same time with the same extract(P<0.05).

**Conclusion:** Green tea injected into sulcular had beneficial antioxidant effect, thus green tea can be used safely and successfully in periodontal treatment.

**Key words:** Green tea extract, oxidative stress, periodontal health. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):112-117).

## INTRODUCTION

The tea plant (*Camellia sinensis* L.) is grown in about 30 countries worldwide <sup>(1)</sup>. Tea (*Camellia sinensis*) is the most widely consumed beverage worldwide for its desirable aroma, taste and putative positive physiological functions <sup>(2)</sup>.

Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxy radicals, hydroxyl radicals, and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage <sup>(3)</sup>. Catechin compounds are hypothesized to help protect against these diseases by contributing, along with antioxidant vitamins (i.e., vitamins C and E) and enzymes (i.e., superoxide dismutase and catalase), to the total antioxidant defense system <sup>(4)</sup>. In vivo studies showed that green tea catechins increase total plasma antioxidant activity <sup>(5)</sup>. Intake of green tea extracts also increases the activity of superoxide dismutase in serum and the expression of catalase in the aorta. These enzymes are implicated in cellular protection against reactive oxygen species <sup>(6)</sup>. This action is combined with direct action on oxygen species by a decrease in the nitric oxide plasma concentration <sup>(7)</sup>.

Malondialdehyde (MDA), a marker of oxidative stress, also decreases after green tea intake <sup>(5)</sup>. These results suggest that catechins could have a direct (antioxidant) or indirect (increase of activity or expression) effect.

Since catechins can act as antioxidants in vitro, they might prevent the oxidation of other antioxidants, such as vitamin E. However, ingestion of green tea catechins does not modify the plasma status of vitamins E and C in vivo <sup>(8)</sup>. This study is directed to evaluate the antioxidant effect of local injection of green tea into rabbit periodontium through estimation the levels of serum MDA and plasma vitamin C. The aim of this study was to assess the antioxidant effect of sulcular injection of green tea to evaluate its use in periodontal treatment.

## ANIMALS AND METHOD

### Study area and animal

This study was carried out at the Hawler Medical University, College of Dentistry, Department of Periodontology and department of basic science, Erbil city during the period from 1<sup>st</sup> Jan 2012 up to 30<sup>th</sup> May 2012. Fifty five (55) 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. The animals were divided into three intervals subgroups, the control (non- injected group), 5%green tea extract (test group) and the Distilled water (extraction solvent) group as shown in (Table 1). Sulcular injection technique was used through the labial gingival tissue of lower right central incisor. The depth of

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penetration was measured by adjusting the sleeve to stop 5mm from the tip of the needle<sup>(9)</sup>. Test rabbit group was injected with 50 µL/Kg<sup>(10)</sup> of

5% green tea extracted in distilled water solution, while the base line control group was injected with distilled water alone.

**Table 1: Classification of the studied groups used for biochemical assay**

No. of rabbits	Time intervals of sample after injection (hr)	Groups		
		Control( non-injected group)	5%green tea extract injection group(test group)	Distilled water injected group
5	-	A	-	-
5	1	-	B1	C1
5	3	-	B2	C2
5	24	-	B3	C3
5	72	-	B4	C4
5	168	-	B5	C5
Total of no. of rabbits	-	5	25	25

### Preparation of 5% green tea extract

Green tea was purchased from supermarket, dry Chinese green tea –Temple of Heaven Gun power, the green tea samples was expired at least one year later. Five (5) g of the selected green teas leaf, 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<sup>(11)</sup>.

### Blood sample Collection

Cardiocentesis blood samples were collected after an overnight fast (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.

### Measurement of Oxidative Stress parameters

#### Serum Malondialdehyde (MDA)

Malondialdehyde (MDA) levels were determined by the MDA thiobarbituric acid (TBA) test using NWK-MDA01 assay kite/Northwest/USA. The test depends on the colorimetric reaction of MDA and TBA in acid solution. MDA, a secondary product of lipid peroxidation, reacts with thiobarbituric acid (TBA) to generate a red-coloured product, which was detected spectrophotometrically at 535 nm. The absorbance of the mixture was measured at 535 nm with a spectrophotometer and the results were expressed as µmol/l.

#### Serum vitamin C

Vitamin C was determined by the method of Stanly<sup>(12)</sup>. Ascorbic acid in plasma is oxidized by Cu (II) to form dehydroascorbic acid, which reacts with acidic 2,4- dinitro-phenylhydrazine to form a red dihydrazone which is measured at 520 nm with a spectrophotometer

### 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.

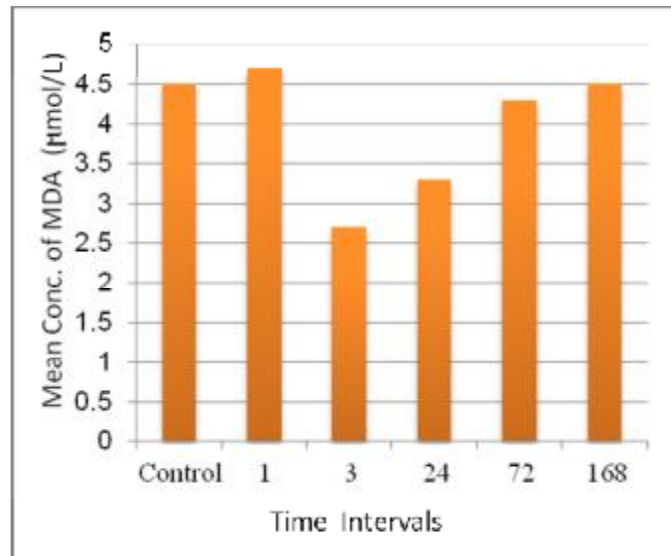
## RESULTS

### Lipid peroxidation (Serum malondialdehyde levels):

Table 2 and figure 1 show the mean and standard error (±SE) of control group (A) and groups after time intervals of intra sulcular injection with 5% green tea extract. The results showed that there is a significant decrease (P<0.05), only 3 hrs after green tea injection (groupB2), while the value was not changed significantly in the other groups those injected with green tea injected extract.

**Table 2: The concentrations of serum Malondialdehyde (MDA) in groups after time intervals of sulcular injection with 5% green tea extract and non-injected rabbit (control) group.(P<0.05)**

Rabbit Groups	No. of Rabbits	Time Intervals (hours)	Mean Conc. of MDA (µmol/L)	±SE	Sig.
A	5	Control	4.5	0.9	--
B1	5	1	4.7	1.48	N.S
B2	5	3	2.7	0.62	S
B3	5	24	3.3	1.39	N.S
B4	5	72	4.3	1.34	N.S
B5	5	168	4.5	1.06	N.S



**Figure 1: The concentrations of serum Malondialdehyde (MDA) in groups after time intervals of sulcular injection with 5% green tea extract and non-injected rabbit (control) group (P < 0.05).**

The effect of distilled water (the extraction solvent) injection into sulcus on rabbit serum MDA is presented in Table (3). The results showed that there was no significant difference in mean concentration values between control group(A) and groups injected with distilled water during time interval(P>0.05).

**Serum Ascorbic acid (vitamin C) in injected groups:**

Table 4 and figure 2 show the mean and standard error ( ±SE) for serum ascorbic acid in rabbit groups injected with of 5% green tea extract into their sulcus. The results showed that vitamin C increased significantly in green tea injected groups comparing to control group, reaching the maximum concentration (0.218± 0.051mg/100g) after three hours of injection (group B2) (P<0.01), then the concentration dropped to control value, 24 hours after green tea extract injection (0.088±0.0164 mg/100g).

The effect of sulcular injection of distilled water (the extraction solvent) on rabbit serum vitamin C is obtained in Table (5). The results showed that there was no significant difference in mean concentration values of serum ascorbic acid between groups injected with distilled water

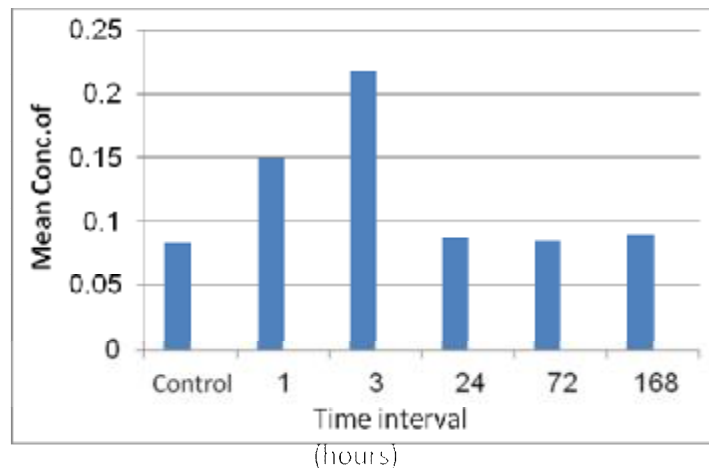
during time interval after injection and control group (P >0.05).

**Table 3: The concentrations of serum MDA in rabbit groups after time intervals of sulcular injection with distilled and control group (A) (P>0.05) .**

Rabbit Groups	No. of Rabbits	Time Intervals (hours)	Mean Conc. of MDA (µmol/L)	±SE	Significance
A	5	Control	4.5	0.9	--
C1	5	1	4.6	1.32	N.S
C2	5	3	4.4	0.63	N.S
C3	5	24	4.5	1.1	N.S
C4	5	72	4.1	0.76	N.S
C5	5	168	4.5	1.30	N.S

**Table 4: The concentrations of serum vitamin C level in rabbit groups after time intervals of sulcular injection with 5% green tea extract and control group ) (P<0.01)**

Rabbit Groups	No. of Rabbits	Time Interval (hours)	Mean Conc.of Vit. C (mg/100 g)	± SE	Significance
A	5	Control	0.084	0.0212	--
B1	5	1	0.17	0.040	S
B2	5	3	0.218	0.051	H.S.
B3	5	24	0.088	0.0164	N.S
B4	5	72	0.086	0.0459	N.S
B5	5	168	0.090	0.0673	N.S



**Figure 2: The concentrations of serum vitamin C level in rabbit groups after time intervals of sulcular injection with 5% green tea extract and control group ( $P>0.01$ ).**

**Table 5: The concentrations of serum vitamin C level in rabbit groups after time intervals of sulcular injection with distilled water and control group**

Rabbit Groups	No. of Rabbits	Time Intervals (hours)	Mean Conc. of vit C (mg/100gm)	$\pm$ SE	Sig.
A	5	Control	0.084	0.0212	
C1	5	1	0.090	0.034	N.S
C2	5	3	0.107	0.077	N.S
C3	5	24	0.108	0.084	N.S
C4	5	72	0.103	0.043	N.S
C5	5	168	0.086	0.022	N.S

## DISCUSSION

### Lipid peroxidation in injected rabbit groups (Serum malondialdehyde levels):

The serum MDA level decreased only 3hrs after sulcular injection with 5% green tea extract with distilled water, while the sulcular injection of distilled water alone had no effect on serum MDA level in the rabbit.

The above results demonstrated that, the sulcular injection of 5% of green tea extract may have a significant role in oxidative stress reactions. This may be due to catechins compounds that are presents in green tea which, can act a scavenger for free radicals that is produced from reactive oxygen species, thus decreasing or preventing cell damage that caused by free radicals<sup>(13)</sup>.

Lipid peroxides, derived from poly saturated fatty acid, are unstable compounds that can decompose to form complex series of compounds. These compounds include reactive carbonyl compounds, of which the most abundant is MDA, a commonly used indicator for lipid peroxidation, oxidative stress, and subsequent cellular injury in the cell and tissue<sup>(14)</sup>.

Silan *et al.*<sup>(15)</sup>, showed that green tea extract blocked cellular inflammatory process

as indicated from alleviation of perivascular edema and reduction in mononuclear leukocytes inflammatory cells infiltration. Green tea extract was able to normalized the elevated lipid peroxide (Thiobarbituric acid reactive substance) level and completely block lipid peroxidation.

The study of<sup>(16)</sup>, showed a significant beneficial changes in lipid peroxidation after green tea drinking (decrease in MDA after green tea drinking).

Augustyniak *et al.*,<sup>(17)</sup> found that green tea decreased the oxidative stress; in addition to that, the administration of green tea increased the activity of superoxide dismutase (SOD) and glutathione peroxidase (GSH Px).

The results obtained in the present study indicated that a single dose of sulcular injection of 5% green tea extract has a significant effect to reduce MDA level in sera rabbit(decrease lipid peroxidation , oxidative stress and cellular injury that caused by free radicals) .

Thus from the present study one can conclude that green extract injection into sulcus had beneficial effect, therefore green tea can be used safely and successfully in oral field.

### Serum Ascorbic acid in injected rabbit groups:

In this study the serum vitamin C increased after subcutaneous injection of 5% green tea extract into rabbit's sulcus. The increase in the mean concentration level started directly after 1 hour of local injection, then reached maximum level, 3 hours after injection, then the level declined and nearly returned to control value after 24 hours and remain till 168 hours after injection. The results indicated that the injection of distilled water (the extraction solvent) had no effect on vitamin C level in rabbit sera.

The oral injection of green tea into sulcus of rabbits, showed an improvement in the antioxidant status of plasma, considering that catechin supplementation has't only a direct action on antioxidant stress, but also it has a direct action, throughout increasing the levels of other antioxidant compounds (such as vit C) normally present in human serum<sup>(18)</sup>.

Green tea rich in polyphenols in addition to ascorbic acid and minerals, these compounds could increase the green tea polyphenol antioxidant directly by chelate metal ions to prevent their participation and indirect by inhibition of prooxidant enzyme. In addition, induction of antioxidant enzymes such as superoxide dismutase which had the ability to convert superoxide radicals into hydrogen peroxides which then metabolites by catalase (CAT)<sup>(19)</sup>.

Hajimahmoodi *et al.*,<sup>(20)</sup> reported that Chinese green tea had more potential antioxidant power comparable with Ahmed green tea. An antioxidant capacity was strongly correlated with the total phenolics content of the tea. It was published that green tea had reduced lipid peroxidation and caused an increase in the activity of antioxidant enzyme in diabetic rats<sup>(21)</sup>.

The potential protective role played by green tea against injurious effect of reactive oxygen species, was studied in human micro vascular endothelial cells. The result showed that green tea polyphenol can acts as a biological antioxidant in a cell culture experimental model and prevent oxidative stress –induced cytotoxicity in endothelial cells<sup>(22)</sup>.

From the results of the present study, one can conclude that the increase in serum level of vit C after single dose of green tea injection into intrasulcular may be due to the presence of this vitamine in green tea.

The present study suggests that there is an association between the intake of green tea and periodontal health condition. One can conclude that the increase in serum level of vit C after

single dose of green tea injection into sulcus may be due to the presence of this vitamine in green tea. From the present study one can conclude that green tea injection into sulcus had beneficial effect, thus green tea can be used safely and successfully in oral field. Green tea sulcular injection could be used as a drug of choice in treatment of periodontal disease

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