# Oral Health & Dental Science

# The Effect of Green Tea Varnish in Comparison with Fluoride Varnish on Remineralization of Artificial Caries

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# ABSTRACT

**Introduction:** Green tea is a natural herb, that has catechin, theanine and amino acids with antioxidant, antiinflammatory, antimicrobial and anti-mutagenic properties that can improve the salivary parameters and consequently protect the oral cavity from diseases. So, the study aimed to investigate the effect of green tea varnish on remineralization of artificial caries and compare its effect with fluoride varnish.

*Materials and Methods:* 30 extracted sound teeth were kept in demineralizing solution for 4 days, to artificially form caries-like lesions. The teeth were divided equally and randomly into three groups. Group 1 was treated with green tea varnish, group 2 was treated with sodium fluoride varnish, and group 3 left without treatment. After treatment pH cycling was done.

**Results:** The mean Ca weight% of green tea and fluoride varnish groups  $(36.85 \pm 0.59 \text{ and } 37.51 \pm 0.65 \text{ respectively})$  were significantly higher than the control group  $(26.98 \pm 0.24)$ . Also, the mean p weight% was found to be significantly higher in the green tea group  $(17.50 \pm 0.36)$  and fluoride group  $(18.31 \pm 0.40)$  versus the control group  $(13.91 \pm 0.15)$ .

*Conclusions:* Green tea varnish had the same remineralizing effect as Fluoride varnish.

**Recommendations:** More studies are required using other concentrations of green tea varnish and may use more than one application, and in vivo studies need to be done.

#### Keywords

Remineralization, Fluoride varnish, Green tea varnish, Dental caries prevention.

# Introduction

Demineralization is the process of removing tooth enamel minerals, and restoring these minerals is called remineralization. When the rate of demineralization surpasses the rate of remineralization, calcium and phosphate dissolve from the enamel, leading to the softening of the tooth structure, over time, if demineralization continues, it can result in the formation of cavities. Demineralized enamel is restored by remineralization which is accelerated when

saliva is saturated with minerals with the help of remineralizing agent [1].

Fluoride is the most commonly used remineralizing agent. It raises salivary pH and prevents enamel dissolution. When fluoride comes into contact with the enamel, it interacts with the OH- groups of hydroxyapatites, transforming it into Fluro-hydroxyapatite, which is more stable and increases the hardness of the tooth enamel [2]. However, fluoride varnish is still a chemical product that may have some drawbacks. American Dental Association reported in 2021, that fluoride varnish may cause swelling, burning, itching, soreness, vomiting, difficulty breathing, tightening of the throat,

and also may lead to loss of consciousness [3].

Green tea is a natural herb, that has catechin, theanine and amino acids together with their antioxidant, anti-inflammatory, antimicrobial and anti-mutagenic properties can improve salivary parameters and consequently protect the oral cavity from diseases such as; dental caries, gingivitis, periodontitis, oral inflammations and oral cancers [4]. The main polyphenol found in green tea is epigallocatechin-3-gallate. This catechin has a strong potential against carcinogenesis, angiogenesis, and tumor metastasis [5].

A systematic review in 2017, analyzed the published articles which measured the efficacy of green tea with different products in dental caries prevention. It was found, with the limited available literature on green tea, that it had an inhibitory effect on dental caries. It was recommended that further studies are still needed [6].

Questions regarding the effect of green tea varnish remained indefinite, so the present study was carried out to study the effect of green tea varnish on remineralization of artificial caries and compare its effect with fluoride varnish.

# **Materials and Methods**

The current study was an experimental in vitro trial that included 30 extracted sound teeth. The study was conducted after approval of the research ethics committee at Pharos University registration no (04-2022-11-27-3-048). The teeth were examined visually using air drying for 5 sec with no evidence of caries, which met a score of 0 in the International Caries Detection and Assessment System (ICDAS) [7] and were included in our study. Teeth with caries, abrasions, cracks, fractures, fluorosis, or developmental defects were excluded.

#### **Samples Preparation**

The extracted teeth were cleaned and decoronated at the level of the cementoenamel junction. A nail varnish was used to cover the entire surface of the crowns of each tooth, leaving a window measuring  $3\times3$  mm. The teeth were stored in distilled water at room temperature until use [8].

#### Preparation of the demineralizing solution

The demineralizing solution contained 2.2 mM  $CaCl_2$ , 2.2 mM  $KH_2PO_4$ , and 50 mM lactic acid pH at 4.4 with a 1M KOH solution [8].

# Preparation of the remineralizing solution

Remineralizing solutions that contained 1.5 mM  $CaCl_2$ , 0.9 mM  $Na_3PO_4$ , and 0.15 M KCL, with a pH of 7.0 was freshly prepared [8].

Remineralizing and demineralizing solutions were prepared in the Faculty of Pharmacy, at Alexandria University.

#### Green tea varnish preparation

To prepare 5% green tea varnish, 100 mg of the epigallocatechin-3-gallate (EGCG) powder, as a main component of green tea

#### **Artificial caries formation**

The teeth samples were kept in 40 ml of demineralizing solution for 4 days, to artificially form caries-like lesions. Then the teeth were removed from the demineralizing solution and rinsed thoroughly with deionized water [11]. The teeth were re-examined visually using ICDAS to be sure that all the teeth have code 1: Opacity or discoloration white is visible at the enamel surface after prolonged air drying [7].

#### Grouping

The teeth were divided equally and randomly into three groups with 10 teeth in each group.

**Group 1:** The teeth samples were treated with 5% green tea varnish once.

**Group 2:** The teeth samples were treated with 5% sodium fluoride varnish once.

Group 3: The teeth samples left without treatment.

# Treatment

The applications of the green tea and fluoride varnishes were done once at the beginning of the study. The varnishes were applied on the artificial caries lesions of the samples of groups 1 and 2 using a brush, then the researcher waited for five minutes for the evaporation of the solvent [12].

# pH cycling

After treatment pH cycling was done to mimic the dynamic demineralization and remineralization process that exist in the oral cavity. All the samples were immersed in 30 ml of demineralizing solution for 3 hours, and 21 hours in remineralizing solution. After each step, the samples were washed thoroughly with deionized water. The demineralizing and remineralizing solutions were replaced every 24 hours. This cycle of demineralizing-remineralizing was continued for 14 days [13].

#### **Evaluation**

After 14 days the samples were evaluated using the scanning electron microscope (SEM) Energy Dispersive Analysis X-ray (EDAX) at the Faculty of Science, Alexandria University to measure the calcium (Ca) and phosphorus (P) weight % of the enamel samples.

#### **Statistical analysis**

Data was collected, tabulated and analyzed using SPSS version 25. The One-Way ANOVA test was used to compare between 3 groups, when significance was found, it was followed by the posthoc test to find significance between pairs.

# Results

Ca and P weight% were determined by SEM-EDAX in the 3 groups (Figure 1).



(a: green tea varnish group, b: Fluoride varnish group and c: control group) Figure 1: SEM-EDAX analysis of the enamel samples of the 3 groups after treatment.

The results found as shown in table 1, that the teeth treated with green tea and fluoride varnish had significantly higher mean Ca weight% ( $36.85 \pm 0.59$  and  $37.51 \pm 0.65$  respectively) than the control group ( $26.98 \pm 0.24$ ), with no significant difference between green tea and Fluoride varnish.

 Table 1: Comparison between the 3 treatment groups regarding Ca weight%.

Treatment groups	Ca weight% Mean ± SD	P value
Green Tea	$36.85 \pm 0.59^{\scriptscriptstyle 1}$	
Fluoride	$37.51 \pm 0.65^{1}$	0.000*
Control	$26.98 \pm 0.24^2$	
* 6: :0 1:6	10.05	

\*: Significance difference p  $\leq 0.05$ 

Different superscript numbers represent significant differences between pairs.

In table 2, when comparing the 3 groups regarding mean p weight% it was found that there was no statistically significant difference between the teeth treated with green tea  $(17.50 \pm 0.36)$  and those treated with fluoride varnish  $(18.31 \pm 0.40)$ . However, the mean p weight% of the 2 groups was significantly higher than control group  $(13.91 \pm 0.15)$ .

 Table 2: Comparison between the 3 treatment groups regarding P weight%.

Treatment groups	P weight % Mean ± SD	P value
Green Tea	$17.50 \pm 0.36^{\scriptscriptstyle 1}$	
Fluoride	$18.31 \pm 0.40^{1}$	0.000*
Control	$13.91 \pm 0.15^{\scriptscriptstyle 2}$	

\*: Significance difference  $p \le 0.05$ 

Different superscript numbers represent significant differences between pairs.

#### Discussion

Polyphenols of green tea have preventive effects on dental caries. It has been suggested that green tea reduces the acidity of saliva and enhances remineralization [14]. As EGCG is considered the most active component of green tea [10], it has been used as the green tea extract in the current study to compare its remineralizing effect with fluoride varnish as the gold standard.

The current study showed that the application of green tea varnishes once at the beginning of the study has the same significant remineralizing effect as fluoride varnish when compared with the control group. This result is consistent with the results of an in vitro study that examined the effect of fluoride varnish on root caries, and it was found that the application of green tea varnishes prevented the development of caries [10]. Another study was conducted to evaluate the effect of green tea varnish on children's salivary parameters, and the results found that green tea varnish increase significantly pH and buffering capacity of saliva and resists the demineralization of enamel [4]. These results may be due to the effect of green tea in maintaining the alkalinity of saliva. The catechins present in green tea represent a marked effect on the pH of saliva. Green tea catechins buffer the acidity and preserve pH within the normal range [15,16].

Another study was conducted to study the impact of green tea on enamel remineralization of immersed teeth in Pepsi. It was found that the application of green tea significantly reduced the enamel roughness and improved enamel remineralization [17]. These results may be explained by the action of green tea catechin which maintains the enamel collagen in an expanded state and the interfibrillar spaces are kept open, this process increases the enamel ability for remineralization [18].

# Conclusions

Green tea varnish had the same effect as fluoride on enamel remineralization and it was significantly higher than the control group.

# Recommendations

This study was an *in vitro* study, so, it was difficult to perfectly simulate the clinical setting. Clinical studies are required to confirm the current achieved results. More studies are recommended to use other concentrations of green tea varnish or more than one application. Green tea varnish can be used as an alternative to fluoride varnish in preventive dental clinics.

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