ORIGINAL ARTICLE

# Effect of Green Tea (*Camelia sinensis*) Extract Gel Concentration of 5% and 10% on Odontoblast Cells after Extracoronal Bleaching with 40% Hydrogen Peroxide

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**ABSTRACT**

**Introduction:** Hydrogen peroxide, a bleaching agent for teeth, can reach and damage pulp tissue by diffusion through enamel and dentine structures. Pulp inflammation, in the form of reactive oxygen species (ROS), causes discontinuity of the odontoblast layer. Antioxidants are common ROS suppressants, one of which is polyphenols found in green tea (*Camelia sinensis*). The aim this in vivo study was to determine the effect of 5% and 10% green tea extract gel concentration in odontoblast cells of the Wistar rats' teeth after extracoronal bleaching with 40% hydrogen peroxide. **Materials and methods:** Fifteen molar teeth of male Wistar rats were applied with a bleaching agent on the occlusal surface. The teeth in the control group were rinsed with warm distilled water after bleaching. In the experimental groups, the teeth were applied with 5% and 10% green tea extract gel after bleaching. Wistar rats were sacrificed on the fifth day after treatment, followed by hematoxylin-eosin staining. Histological examinations were observed under light microscope using 400x magnification, followed by determination of discontinuity scoring. **Results:** The results of Kruskal-Wallis test showed a significant effect of the green tea extract gel concentration for the odontoblast layer discontinuity in all groups ($p < 0.05$). Mann-Whitney test result showed significant difference in all compared pair groups ($p < 0.05$). **Conclusion:** The application of 10% green tea extract gel shows the lowest discontinuity score compared to the 5% extract gel application and the control group.

**Keywords:** Green tea, Camelia sinensis, Odontoblast, Bleaching, Wistar rat

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## INTRODUCTION

Tooth bleaching is one of the most popular treatments for tooth discoloration due to its conservative approach, comfort, simplicity, cheapness, and long-lasting results. Hydrogen peroxide ($H_2O_2$) 40% is commonly used for extra-coronal bleaching because of its practical and rapid results (1). However, several side effects of in-office bleaching treatment include irritation, reduced strength of tooth enamel, hypersensitive teeth, roughness in restoration material, and tooth inflammation (2). One of the causes of inflammation in the teeth, reactive oxygen species (ROS), can trigger oxidative stress in the pulp cell and damage cell ,membranes, decrease cell viability, extracellular matrix degradation, and cause cell necrosis (3,4).

Odontoblast layer is the outermost layer of pulp, with its *processus* within the dentin tubule. Odontoblast is the first cell exposed to toxic agents from dental material that diffuses *via* dentin tubules (5). When H2O2 40% releases ROS into the odontoblast, it will cause a reduced number of odontoblast cells, followed by discontinuity and changing its shape to a cuboidal with an irregular predentin layer (6).

Many methods can prevent oxidative stress, such as applying antioxidants. Several studies have been conducted to find herbal-based antioxidants that have non-toxic effects and are safe for oxidized teeth. *Camellia sinensis* (green tea) contains approximately 4000 bioactive components; one-third of them is a polyphenol that consists of catechin, epicatechin gallate (ECG), epicatechin (EC), epigallocatechin (EGC) and epigallocatechin gallate (EGCG). The most found active component in green tea is EGCG, around 50% - 80% of the total catechin. EGCG has antioxidant, anti-inflammation, and anti-carcinogenic effects (7), which has eight hydroxyl (-OH) groups that are effective for binding and removing ROS directly or indirectly with enzymes (8).

## MATERIALS AND METHODS

This study was ethically approved by The Research Ethics Commission, Faculty of Dentistry, Universitas Gadjah Mada with reference number 35/UNI/KEP/FKG-RSGM/EC/2023. In materials and methode section should be divided into 5 sub-section.

### Preparation of 5% and 10% green tea extract gel

Fresh green tea leaves were obtained from Pagilaran Private Company (PT. Pagilaran),Kulonprogo Regency, and kept in the laboratory to make the extract within 24 hours. Green tea extract was obtained by the maceration method, in which green tea leaves were dried in an oven at 50°-60°C for two hours. Dry leaves were ground and soaked in 70% ethanol for 24 hours, mixing them for the first three hours. Ethanol was then removed by a rotary evaporator from the product of maceration filtration to obtain 5% and 10% concentrations of green tea extract. The topical gel was made by mixing 10 ml of gelling agent CMC-Na at 80°C with 5% and 10% green tea extract in glycerin. The mixture was then added to propylene glycol, methylparaben, and water as calculated to obtain the desired homogenous gel.

### Treatment of experimental animal

The research subjects consisted of nine Wistar rats divided into three groups. One group consisted of three wistar rat and were placed in one cage and fed ad libitum. Male were chosen because they were not affected by hormones and were 2-3 months old. The wistar rat. Anesthetized with 40 mg per body weight of mice ketamine-xylazine intramuscularly.

A gingival dam was applied in the fornix and palatal area. The occlusal teeth area was applied with 0.01 ml of $H_2O_2$ 40% (Opalescence Boost PF 40%, Ultradent, USA) until the cusp ridge. The gel was then applied for five minutes and cleaned up with an aspirator tip without using water. Wistar rats were categorized into three groups, each group consists of three rats. Group I (control) only rinsed with 1 ml of distilled water at 50°C for 5 minutes on the occlusal area. Group II the molar of rat tooth were threated using 5% green tea extract by micro brush for 3 minutes. Group III the molar of rat tooth were treated by 10 % green tea extract by micro brush for 3 minutes. The wistar rat's jaw was dissected five days after the experiment with the lethal dose of intramuscular anesthesia with ketamine-xylazine. The jaw and the molar teeth were collected and fixed with 10% buffered formalin for 24 hours.

### Preparation of histology preparation of rat teeth

Decalcification of the jaw and teeth was performed with EDTA solution for 35 days, followed by washing with 70%, 80%, 95%, and 100% ethanol for 90 minutes in the automatic tissue processor (Sakura Tissue-Tek II, Japan). The tissue was washed with xylol I, II, and III for 90 minutes, soaked in liquid paraffin at 57°-59°C for two hours, and waited for 20 minutes to make a paraffin block. The block was sectioned by a microtome (Sakura Accu-cut SRM Microtome, Japan) and soaked in the water bath (Sakura PS-M, Japan) at 50°C and mounted onto the object glass slide (Sail Brand, China). The slides were then put into the slide warmer (Sakura PS-53, Japan) at 40°- 45°C for 15 minutes, followed by deparaffinization with xylol and rehydration with alcohol followed by H&E staining.

### Scoring the continuity of the odontoblast layer

The odontoblast layer was examined in the occlusal area with a light microscope at 400x magnification. Three slides were obtained for each tooth tissue, and three fields of view for the examination. This study classified the odontoblast by three continuity scores (Table I)

**Table I: The criteria of continuity scores in odontoblast histological analysis**

| Score | Characteristics |
|---|---|
| 1 | Normal odontoblast layer, defined as normal continuity between tissues (fine cell-to-cell connection), absence of vacuole, and palisade cell structure. |
| 2 | Loss of continuity of the odontoblast layer, defined as loss of tissue connection in half of the layer, with half of the cells remaining palisade-shaped. |
| 3 | Total discontinuity of the odontoblast layer with irregular-shaped cells. |

### Statistical analysis

This Study obtained the continuity score of the odontoblast layer as ordinal data and analyzed by the Kruskal-Wallis test with a 95% confidence interval ($\alpha$ = 0.05). After we found a significant result in the Kruskal-Wallis test, This study performed the Mann-Whitney test to determine the effect of green tea extract gel in two experimental groups.

## RESULTS

Palisade-shaped odontoblast cells formed a continuous layer located between dentine an pulp. This study defined a continuity score in the odontoblast layer after applying 5% and 10% green tea extract in the wistar rat's molar pulp after extra coronal bleaching using $H_2O_2$ 40%. Histological observation in the odontoblast layer of the molar pulp displayed column-shaped odontoblast cells with a round-shape nucleus polarized toward the basal membrane. In the control group found a loss of continuity in the odontoblast layer with prominent vacuolesand smaller cell sizes (Fig.1). Interestingly, applying 5% and 10% green tea extract improved the continuity of the odontoblast layer (Fig. 2-3).The mode score in the Control Group where warm distilled water was applied after the bleaching application was a score of 3, The group that applied 5% green tea extract gel after bleaching had a mode score of 2, and in the group that applied 10% green tea extract gel after bleaching, the mode score was 1. From the results of this study, an analysis was carried out to determine the effect of green

tea extract gel after application of the bleaching agent on the odontoblast layer. between the three groups using the Kruskal-Wallis Test . The Kruskal-Wallis test, which resulted in $p = 0.001$ ($p < 0.05$). This result suggests that green tea extract significantly affects the discontinuity of the odontoblast layer in the pulp layer after $H_2O_2$ 40% bleaching. The result of Mann-Whitney test and found a significant difference in the odontoblast layer between all compared group pairs ($p < 0.005$) (Fig.4).

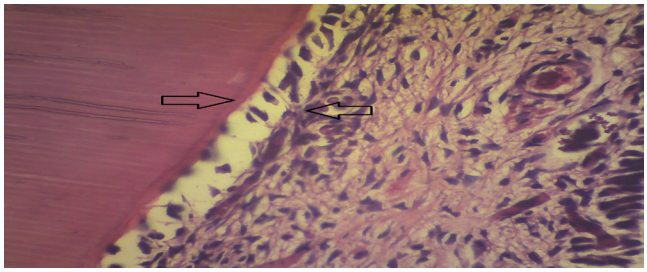

**Fig. 1: Histological observation of discontinuity scoring of odontoblast layer in the rat pulp teeth after application 40% hydrogen peroxide. Odontoblast layer was not observed; only odontoblast cells were observed, which did not form a layer. Also, many vacuoles were observed.**

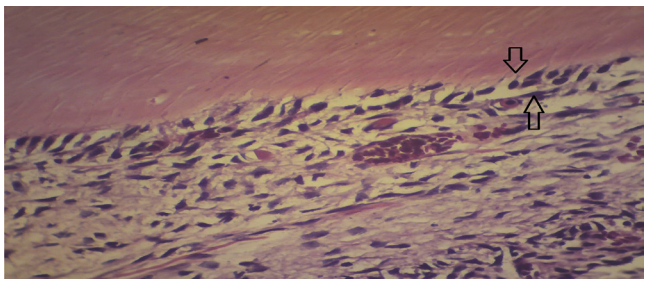

**Fig. 2: Histological observation of discontinuity scoring of odontoblast layer in the rat pulp teeth after application 40% hydrogen peroxide and extract green tea gel 5%, odontoblast layer lost its continuity, and the presence of vacuoles, with some of the odontoblast layer remaining palisade-shape.**

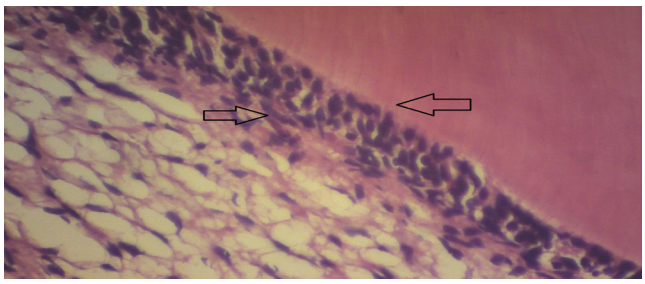

**Fig. 3: Histological observation of discontinuity scoring of odontoblast layer in the rat pulp teeth after application 40% hydrogen peroxide and extract green tea gel 10%, well-organized arrangement of palisade-shaped odontoblast was observed.**

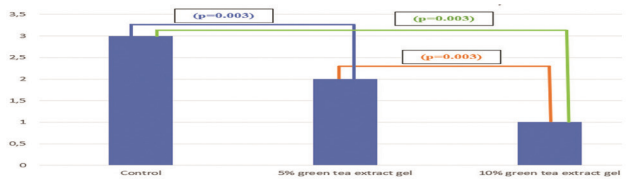


**Fig. 4: Graph of Mode Value and Results of Mann-Whitney test for continuity score in the odontoblast layer after rinsing with warm distilled water, 5% and 10% green tea extract.**

## DISCUSSION

$H_2O_2$ is a strong oxidator agent that is rapid and effective in teeth bleaching. This molecule breaks a heavy chain of chromophores, making it easy for chromophores to diffuse and become colorless (9). However, H O releases a vast amount of free radicals, which cause inflammation in the pulp teeth and vacuolization in the odontoblast cell layer structure (10). Odontoblasts are cells located on the periphery of the pulp chamber, providing the first line of defense against pulp irritation. The penetration of $H_2O_2$ into the pulp chamber caused changes in the odontoblast cell layer, namely the occurrence of vacuolization of the odontoblast cell layer in all groups that were applied with $H_2O_2$. The vacuolization of the odontoblast cell layer in the pulp tissue is the initial response of the odontoblast cells to injury and usually occurs before the pulp becomes inflamed (10). A previous study by (11) demonstrated the role of antioxidants in removing the negative effects of free radicals. Antioxidants bind to the free radicals and transfer their hydrogen ions or electrons for stabilizing and reducing the reactivity of the free radicals. In this study, we used green tea, which is a biocompatible natural antioxidant agent. Green tea is composed of these molecules: gallic acid (GA), (-)-gallocatechin (GC), (+)-catechin (C), (–)-epicatechin (EC), (–)-epigallocatechin (EGC), (–)-epicatechin gallate (ECG), (–)-epigallocatechin gallate (EGCG), p-coumaroylquinic acid (CA), and (–)-gallocationchin-3-gallate (GCG), which is EGCG is the most abundant molecules. The effectiveness of EGCG in green tea for removing free radicals is 100 times more than vitamin C and 25 times more than vitamin E.

The Wistar rat is a widely accepted mammalian model for in vivo experiments (12). Many similarities are found in the human and mouse tooth pulp tissue; thus, Wistar rats can be used to study the inflammation effect in the pulp. Previous in vivo studies of pulp reaction after single bleaching revealed various degrees of inflammation, from mild and acute inflammation to even necrosis in the coronal area of the pulp. Based on a study using the Wistar rat model, applying single-time H O displayed inflammation and necrosis in the pulp horn.

This results demonstrated that rinsing warm distilled water in the control group after applying the $H_2O_2$ 40% caused discontinuity of the odontoblast layer due to the loss of cell-to-cell connection and presence of multiple vacuoles, causing the irregular structure of the odontoblast layer. However, in the 5% and 10% green tea extract gel groups, there was a significant decrease in the number of irregular odontoblast layers, along with improved discontinuity.A previous study by (14) showed that washing with warm distilled water after bleaching cannot completely remove free radicals released from the bleaching agent. X-ray diffraction (XDR) examination revealed the presence of $H_2O_2$ in teeth after bleaching, even after rinsing with warm

distilled water (15). After washing the $H_2O_2$, onasen (one of the free radicals) remains in the enamel prism and dentine. Peroxide and onasen persists in the dentine until eliminated by systemic microcirculation in the pulp. In the experimental groups with 5% and 10% concentrations of the green tea extract gel, we improved the discontinuity score in the odontoblast layer. Green tea contains polyphenols, composed of several chemical molecules, such as flavonol, flavandiol, flavonoid, and phenolic acid, that make up 30% of the weight of the dried tea. Polyphenols also consists of catechins, and there are four main catechins in green tea: Epigallocatechin gallate (EGCG), Epigallocatechin (EGC), Epicatechin gallate (ECG) and Epicatechin (EC). About 59% of the total catechin is the EGCG, followed by 19% of EGC, 13.6% of ECG, and 6.4% of the EC (8). EGCG is the main active constituent of green tea. It is formed by a benzenediol chain that binds to tetrahydropyran fraction, pyrogallol ring, and galloyl group (with pyrogallol). The structural characteristics of the catechins in green tea significantly contribute to the antioxidant effect of green tea. Antioxidants are involved in transferring hydrogen or single electron, or both. Catechin, an antioxidant in green tea, removes lipid radicals and breaks the peroxyl and alkoxyl chains . In addition, polyphenols in green tea prevent the formation of free radicals and neutralize free radicals by electron exchange via dihydroxyl (from chain B) and trihydroxyl groups (16).

This study demonstrated that a 10% concentration of the green tea extract gel reduced the discontinuity score of the odontoblast layer compared to a 5% concentration. This result was supported by more EGCG in the 10% green tea, contributing to more effective binding ROS. EGCG is known to be involved in the inflammation system by inhibiting the migration of the neutrophils via endothelial cells and reducing the number of ROS. It also can clean up ROS directly by binding with ROS or indirectly by reducing ROS and the enzymes (8).

## CONCLUSION

The 10% concentration of green tea extract gel reduce inflammation of the pulp after bleaching by decreasing the discontinuity score in the odontoblast layer than application of 5% green tea extract gel and control group. Further study is needed in a human patient to assess the clinical response of applying green tea extract gel after bleaching.

## ACKNOWLEDGEMENT

We would like to thank the Faculty of Dentistry at Gadjah Mada University for financial support for this research.

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