# Antibacterial Effect of Green Tea Extract Epigallocatechin Gallate against Enterococcus faecalis as Intracanal Medicament (An in vitro study)

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## Abstract

Objectives: The aim of this study was to assess the antibacterial efficiency of various concentrations of green tea extract Epigallocatechin Gallate (EGCG) as intracanal medicament and compared it with the effect of calcium hydroxide paste on *Enterococcus faecalis*. Material and methods: Intracanal medicament was prepared by mixing powder of EGCG extract with glycerol material. Forty human single rooted teeth were prepared, sterilized, cultured with *E. faecalis* and incubated for 24 hours. Specimens were taken from root canal using file and CFU of bacterial colonies were measured. Teeth were classified into 4 groups: negative control group with no intracanal medicament, positive control group with calcium hydroxide paste intracanal medicament, and three tested groups with 2%, 3%, and 4% of prepared EGCG intracanal medicaments. After 3days; the intracanal medicaments were removed, specimens were taken and bacterial colonies were measured by CFU to assess the antibacterial activity in each group. Results: All tested groups showed significant antibacterial effect against *E.fecalis*. EGCG group at 2% showed the least antibacterial effect. While both 3% and 4% EGCG groups showed high antibacterial effect which was similar to that of Ca $(OH)_2$. Conclusion: Green tea catechine (EGCG) can be used effectively as intracanal medicament, specifically at concentration of 3% and 4%.

**Keywords**: **Epigallocatechin Gallate; Intracanal medicament; *Enterococcus faecalis*; Green tea; Enterococci; Endodontic infections**

## Introduction

Primary endodontic infections are caused by oral microorganisms, that originally opportunistic pathogens which may attack a necrotic root canal and start an infectious process. One of the main causes of failure of root canal treatment is reinfection of the persistent microorganism.[1]

*E.faecalis* which is an Anaerobic bacteria has specifically obtained special attention, as it is one of the main microorganisms that found in cases of post endodontic treatment infection.[2] Enterococci can resist very hard conditions like high alkaline pH and salty environment. It can stay alive even in good instrumented and obturated root canals with low nutrients. The ability of Enterococci to resist high pH belong its proton pump which attract protons into the cell and increase the cytoplasm acidity. [1]

Disinfection of entire root canal considers one of the main purposes of endodontic treatment. This purpose could be obtained using mechanical instrumentation, chemical irrigation, and temporary medication of the canal.[3] The chemomechanical root canal preparation can reduce root canal infection, but due to the complicated anatomy of the root canal system, microorganisms may able to survive. So, to complete the disinfection of the entire root canal system, the antimicrobial intracanal medicaments are used. [4]

It is necessary to assess the antimicrobial efficiency of various intracanal medications as the role of root canal medicaments becomes more complicated in endodontic treatment with apical periodontitis because of wide range types of microorganisms. [5]



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Calcium hydroxide Ca $(OH)_2$ is commonly used as an endodontic intra-canal dressing material between root canal treatment appointments, because of its tissue dissolving, antiresorptive and antibacterial properties. [6] However, calcium hydroxide is not efficient in removing bacteria present inside dentinal tubules. It was found that *E. faecalis* that inside the dentinal tubules was resistant to calcium hydroxide over ten days.[7]

Side effects of Synthetic drugs and the constant increase in antibiotic-resistant strains lead to increase demand for using an alternative disinfecting measure. To overcome the disadvantages of the usually used medicaments, use of herbals like green tea as an alternative is suggested. [2]

Green tea is a classical drink in eastern countries like Japan and China which prepared from the young leaves of tea plant Camellia Sinensis. The leaves of the tea plant contain flavonoids which are polyphenolic components have great activity against a wide spectrum of microbes. [8]

Flavonoids which found in green and black teas restrain the activity and growth of the bacteria related with tooth decay occurance. The main polyphenol component of green tea; Epigallocatechin Gallate (EGCG) is responsible for the main green tea health benefits, such as antioxidant, antidiabetic, antimicrobial, anti-inflammatory, protease inhibition effects, and cancer prevention.[9] The anti-oxidant property of unfermented tea could be related to polyphenols ability, especially the gallocatechins, to inactive free radicals.[6]

The purpose of this study was to investigate the efficiency of green tea catechine EGCG as intracanal medicament against *E.fecalis* and compared it with Ca $(OH)_2$ medicament.

## *Materials and Methodologies*

### Samples Preparation

Forty extracted permanent human mandibular premolars with complete apices were collected, the roots surfaces were cleaned by a curette to remove all remnant tissue after extraction, then kept in DL (distilled water) to avoid dehydration. Then all of the teeth were sectioned cervically by diamond disc and standardized at WL (working length) to 11mm for all of the roots.

Protaper manual file system (Dentsply company) was used to prepare the root canals to the full WL until size F3, during instrumentation the canals were irrigated with 1ml of sodium hypochlorite with concentration 5.25% between each file to avoid canal blockage then irrigated with 1ml of 17% EDTA, after that each canal were washed by 2ml of normal saline and dried by using paper points. Then the apical foramen was sealed by cold cure acrylic resin in order to prevent the bacterial leakage and the outer surface of each root was coated by 2 layers of nail-varnish as shown in “Figure 1”. Then the roots were imbedded in Addition silicone impression material was putted in stainless steel box as shown in “Figure 2”. When the impression material completely set, the boxes were covered by foil of aluminum then sterilized by autoclave with cycle to 121oC for 15 minutes. Assurance of sterilization was done by make culturing test with no bacterial growth.

Figure 1: Teeth sealed apically



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Figure 2: Roots imbedded in addition silicone material and covered with varnish

**Preparation of Medicament**

The powder of green tea extract Epigallocatechin Gallate (Teavigo, Swanson health products, USA) was mixed with pure glycerol (inert material) to obtain creamy medicament according to the following concentration by mixing 1ml of glycerol with 0.2gm of EGCG to prepare 2% of medicament and mixing 1ml of glycerol with 0.3gm of EGCG to prepare 3% of medicament and by mixing 1ml of glycerol with 0.4gm of EGCG to prepare 4% of medicament.

The Calcium hydroxide medicament (Metapex, MetaBiomed) which is ready made paste that contain Ca $(OH)_2$ and Iodoform was used in this study.

**Preparation of culture media**

The powder of Enterococcus agar (M-Enterococcus agar, Alpha Biosciences) was added to distilled water in ratio according to manufacture instruction and poured in flask and sterilized by using autoclave at 121° C for 15 minutes and dispensed into petri-dishes under complete a septic condition.

**Preparation of bacterial suspension**

Samples of bacteria was taken from infected root canals during endodontic treatment of randomly treated teeth by using paper points and putted into tubes contain 4ml of Brain heart infusion broth and incubated at 37°C for 24 hrs after that seen turbidity of broth that is mean growth of bacteria. After that 0.5 ml of bacterial suspension was inoculated on selective media (Enterococcus-agar media) then incubated at 37°C for twenty-four hours. The only microorganism that can grow on this media is *E. faecalis*, while other kinds of bacteria cannot grow.

After that a loop full was streaked to remove 5 colonies of *E. Faecalis* form petri-dish of Enterococcus agar medium and putted in 4ml of Brain heart infusion broth and incubated at 37°C for twenty-four hours.

**Root canals inoculation with *E. faecalis***

Each root canal was inoculated with 10µl of bacterial suspension by using pipettes and incubated the root canals at 37°C for 24 hrs. Then the total number of samples (40) was divided into 5 groups (8 samples in each one). Control negative group: (root canals infected with bacteria and not treated), control positive group: root treated with Ca $(OH)_2$ medicament, and three tested groups (roots treated with 3 concentrations (2%, 3%, and 4%) of EGCG medicament paste).

**Application of medicament**

The application of medicaments Ca $(OH)_2$ and EGCG was done by using sterilized protaper file size F2 which saturated with each medicament and inserted to the working length (11mm) of groups of root canals treated with medicament, then samples inside the stainless-steel box capped with foils of aluminum and incubated at 37 °C for 3 days.

**Microbiological sampling from treated root canals**

After each kind of treatment, negative, positive control and tested groups, sample was obtained from each root canal by utilizing sterile protaper file F3 which was fitted inside each canal to the full WL then rotated 360° degree in clock wise direction in order to engage the dentin of the root canal.

All of the samples were carried immediately to tubes contain 1 ml of normal saline and agitated for 30 seconds then 100 µl was cultured on Enterococcus-agar petri-dish, culturing was made by spreading using a loop-full on Enterococcus-agar and incubated at 37°C for



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twenty-four hours. After the incubation period, counting the CFU of the bacterial colonies were done directly on Enterococcus agar.

**Colony-forming units (CFU)**

After the culture was broth, dilution materials were made and then spread out and colonies were counted on the plate using formula[10]:

$$\text{Bacteria per mL} = \frac{\text{(no. of colonies present on the plate) x (dilution factor)}}{\text{culture plated volume}}$$

**The ethical clearance**

Approval for the study carried out on human extracted teeth from Department of Conservative Dentistry, College of Dentistry, University of Mosul under ethical approval no: 2020/011/DM/0002

## Results

The results obtained from counting CFU of bacterial colonies as shown in "Figure 3".

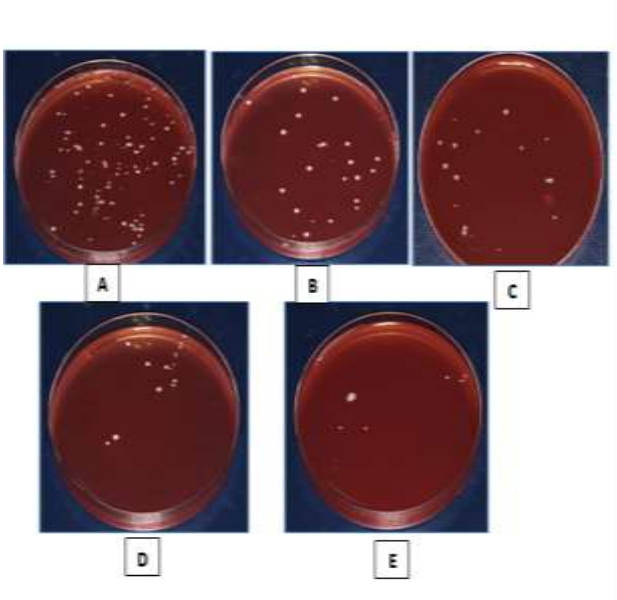


Figure 3: Bacterial colonies on enterococcus agar. A, control group (no treatment). B, 2% EGCG group. C, 3% EGCG group. D, 4% EGCG group. E, Ca (OH)2 group.

They were statistically significant using one way ANOVA ($P$ <.0001) (see Table 1), followed by Duncan Multiple range test (see Table 2). The result of this study revealed that there was a significant antibacterial effect for all experimental groups against *E. fecalis*. Ca $(OH)_2$ group showed the highest mean of antibacterial effect followed by both 4% and 3% EGCG groups with no significant difference between them. 2% EGCG group showed the least antibacterial effect but still effective significantly against *E. fecalis* when it compared with control group. No significant difference was detected between 2% and 3% EGCG groups.

Table 1: One Way ANOVA results for antibacterial effect of different concentration of green tea extract Epigallocatechin Gallate against Enterococcus faecalis

| | Sum of concentration | df | Mean Concentration | F | P value |
|---|---|---|---|---|---|
| **Between Groups** | **5072.5** | **4** | **1268.125** | **10.55** | **0.0001** |
| **Within Groups** | **4206.875** | **35** | **120.196** | | |

Table 2: Duncan's Multiple Range Tests for antibacterial effect of different concentration of green tea extract Epigallocatechin Gallate against Enterococcus faecalis

| Group | CFU/ml Mean ± SD | Duncan 's test |
|---|---|---|
| **Group Ca $(OH)_2$** | **$1.5 \times 10^2 \pm 0.4 \times 10^2$** | **A*** |
| **Group EGCG 4%** | **$4.5 \times 10^2 \pm 1.7 \times 10^2$** | **A** |
| **Group EGCG 3%** | **$8 \times 10^2 \pm 3.1 \times 10^2$** | **AB** |
| **Group EGCG 2%** | **$20 \times 10^2 \pm 3.4 \times 10^2$** | **B** |
| **Group Bacteria** | **$40.5 \times 10^2 \pm 7 \times 10^2$** | **C** |

* Different letters mean significant differences



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## *Discussion:*

Several kinds of bacteria were recovered from cases with of endodontic failure. *Enterococcus faecalis* is gram-positive facultative anaerobic cocci was found to be the most prevalent bacteria. This high prevalence is related to risk factors like gelatinase, enter-ococcal surface proteins, aggregation substances, capsular polysaccharides, toxic cytolysin, generation of extracellular superoxide and antibiotic-resistance determinants. Virulence factors could enhance the adherence of hosts' cells and extracellular matrix, immunomodulation effect, invasions of the tissue, because damaging effect of toxin.[11] The pathogenicity may related to its′ capability to the production of cytolysin, which cause distruction of target membranes like bacterial cells, erythrocytes, and other cells mammalian.[1]

For this reason *E.fecalis* was chosen in this study as a test organism. Furthermore, *E. fecalis* is non-fastidious, easy to culture, can rapidly and efficiently colonizes dentinal tubules in vitro and it had been utalized successfully in prior studies. *E.fecalis* has been detected in ninety percent of teeth with endodontic failure .[5]

The success of root canal treatment can be increased significantly by using biocompatible intracanal medicament between appointments which should possess antibacterial properties to decrease and get rid of bacteria in the system of the root canal. [12]

In this study, Calcium hydroxide Ca $(OH)_2$ was utilized as a "positive control group" because it still the standard widely used intracanal medicament all over the world.[13] The beneficial antibacterial properties of Ca $(OH)_2$ have been attributed to its high alkaline pH (11-12.5) and its decomposition to the lethal, extremely interactive hydroxyl ions. That kill bacterial cells by damaging the cytoplasmic membrane., DNA damage and denaturation of protein.[14] Furthermore, its physical presence prevent root canal reinfection by preventing the ingress of bacteria either coronally or apically, preventing the nourishment, of the residual bacteria, so the recontamination will be delayed.[15] These findings agree with the results of this study as the group of Ca $(OH)_2$ showed the best antibacterial effect against *E.fecalis*.

Recently, herbals and natural products become more popular in medical and dental practice because of their antimicrobial activity, biocompatibility, antioxidant and anti-inflammatory properties. herbal medicine (according to WHO) is a material derived from plant which has or processed ingredients with therapeutic values.[6] Green tea which is made from unfermented leaves, is a rich with polyphenols, particularly flavonoids. Polyphenols in tea are existing in the form of catechins like: epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate [usually named as EGCG]. Catechin constitutes nearly thirty percent of the dry leaf weight and is responsible for several biological properties of the green tea.[1] It is found that Catechins has inhibitory action against *S. mutans* and *S. sobrinus*, with MICs between fifty and one thousand μg /ml. It can prevent the pathogen of oral streptococcuss from being attached to oral surfaces. The catechins also can reduce the caries process by inhibiting streptococcal glucosyl transferase enzyme. [16]

The most active catechin in green tea is EGCG, which is a bitter taste, water soluble component that have wide biological activities like anti-inflammatory, antioxidant and scavenging activity of free radicals. [9] For this reason EGCG was chosen to detect its antibacterial activity. It may have an added advantage to be used as intracanal medicament.

In this study, antibacterial activity of green tea catechine (EGCG) at concentration of 3% and 4% was comparable to that of Ca $(OH)_2$ which consider the standard for comparison as root intracanal medicament. EGCG at concentration of 2% showed the minimum mean of antibacterial effect. However, it still had significant antibacterial effect when compared with control negative group (bacteria group).

The results of this study agreed with the result of Martine et al., 2013 which obtained significant antibacterial effect of EGCG at concentration of 2.5% and 3% comparable to



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that of chlorhexidine when it is used as irrigation solution.[11] Also in a study of Prabhakar et al (2010) that used green tea polyphenols, significant antibacterial activity have been found against biofilm of the *E. faecalis*.[8]

EGCG affects cellular processes which depend on cell membrane like signaling, cell cycle, metabolism of arachidonic acid, and function of mitochondria. The structure of bacterial cell membrane has a lipid bilayer form that EGCG is bound to it and result in lipid vesicles aggregations so the contents are leaked from vesicles suspention.[17] Expansion of the cell membrane is occurred which lead to its thinning, cell structure is lost, and all these factors lead to bacterial cell death. EGCG also inhibit the replication process of DNA because it inhibits bacterial DNA gyrase enzyme. The high efficiency of catechins in green teas' extracts can be explained by the ability of its benzopyran ring to penetrate deeply to the active position and the galloyl moiety anchors it to the cleft by the interaction with its′ hydroxyl(OH)group.[18] Shabbir and his colleagues assessed the impact of demographic factors, and the type of the tooth on post endodontic pain in teeth with necrotic pulp and periapical periodontitis(symptomatic) with presence of radiolucency dressed with Ca(OH)2 or propolis paste randomly, and they concluded there was no real impaction. [19] Others used different materials like hydrogel-based antibiotic intracanal medicaments [20], NF-kB and collagen type 1 [21], alpha-tocopherol [22], and CH plus chlorhexidine [23] as antibiotics in intracanal medicaments.

Gougousis et al., concluded that the activity of calcium hydroxide showed better outcome in respect the number of stem cells on surfaces of the root canal.[24] Whereas Ledermix as intracanal medicament had minimum inter-appointment pain.[25]

Some authors found that Ca (OH)2 preparations had high pH around the roots after seven and ten days and Metapex remained to has higher pH after fourteen days.[26]

Mehta and their colleagues compared the antimicrobial activity of triple antibiotic paste (TAP) and a proton pump inhibitor (PPI) in combination with Ca (OH)2 against *Candida albicans and E. faecalis.* They noticed that TAP have best antibacterial features than PPIs. [27]

## *Conclusions*

green tea catechin EGCG showed acceptable antibacterial effect against *E. faecalis*, with no significant difference between its 3%, 4% concentrations and Ca $(OH)_2$. So, the use of green tea catechin EGCG as intracanal medicament, particularly at concentration of 3% and 4%, could be considered. But still the Ca $(OH)_2$ is superior than all concentrations of green tea catechin extract.

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