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Comparison of the Antimicrobial Efficacy of Green Tea Extract With 1% Sodium Hypochlorite Against *Enterococcus faecalis*: An In Vitro Study

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Abstract

Background: Residual microorganisms are the main cause of root canal treatment failure. Due to the occurrence of bacterial resistance against antibiotics and the side effects of these drugs, herbs that possess antimicrobial qualities are drawing more attention than before.
Objectives: The aim of this study was to compare different concentrations of green tea with 1% sodium hypochlorite with regard to their antimicrobial effects on *Enterococcus faecalis*.
Materials and Methods: In this experimental study, extracts of green tea with four different concentrations (40, 80, 100 and 200 mg/mL) were prepared. *E. faecalis* was cultured on Mueller Hinton agar. Within each culture plate, a disc with one of the concentrations of green tea extract, a disc of 1% sodium hypochlorite, a disc of tetracycline as a positive control, and a disc of distilled water as a negative control was placed. After 48 hours, the mean of the inhibition zone diameter was compared by using one-way ANOVA.
Results: The mean of the inhibition zone diameter around the discs of green tea extract in all concentrations was significantly less than 1% sodium hypochlorite ($P < 0.001$). The antimicrobial effect increased in higher concentrations of extracts of green tea ($P < 0.001$).
Conclusions: Although the antimicrobial effect increases in higher concentrations of extracts of green tea, 1% sodium hypochlorite has a greater antimicrobial effect on *E. faecalis* in comparison with extracts of green tea.

Keywords: Root Canal, Irrigants, Green Tea, Sodium Hypochlorite, Disk Diffusion, Antimicrobial Test, *Enterococcus faecalis*

1. Background

One of the most important factors in the failure of endodontic treatments micro-organisms that remain in the complex system of the root canal and periradicular area (1). The success of endodontic treatment depends largely on the removal of micro-organisms from the root canal system. Since pulp and periapical infections are of bacterial origin and the success of endodontic treatment is directly affected by the removal of micro-organisms canal (2), complete removal of necrotic and infected substances from the root canal is important. During root canal treatment, large amounts of bacteria are removed from the canal by cleaning. But many canals have such an anatomical complexity that even with meticulous cleaning, micro-organisms will be left in the canal. In fact, on average only 50% of the bacteria are removed from the canal (3, 4). Therefore, at the time of canal preparation, irrigants and substances should be used so that by removing debris and necrotic pulp tissue, more micro-organisms can be eliminated. So, irrigants play a critical role in determining the success of endodontic treatment (5).

Enterococcus faecalis is one of the types of bacteria that is resistant to different antibiotics, and several studies have shown that this bacterium is one of the most common species that is separated from the canal after treatment (6). Sundqvist et al. (7) identified the presence of *E. faecalis* on 38% of the teeth which could not be treated during their previous treatment. In addition, only 33% of the teeth that contained *E. faecalis* at the time of replenishing had been successful after re-treatment. Therefore *E. faecalis* is an important cause of treatment failure and its presence at the time of root canal filling reduces the treatment success rate. These bacteria can invade the dentinal tubules, and can survive the activities of endodontic instruments and irrigant substances. Unlike species that cause the initial root canal diseases, *E. faecalis* is also colonized in single-species infections. This means that it does not need the food produced by other bacteria and grows without the aid of other micro-organisms within the canal. Therefore, it is one of the most important and stable factors in endodontic treatment failure (8, 9).

E. faecalis is resistant to several antibiotics, including clindamycin, aminoglycosides, beta lactamase antibiotics, and calcium hydroxide with low levels of concentration.

Therefore it can produce biofilm and cause treatment failure in canals treated with calcium hydroxide (10). In order to cope with *E. faecalis*, different substances such as calcium hydroxide, chlorhexidine potassium iodide, various antibiotics, laser, ozone, and sodium hypochlorite are used (11-13). However, *E. faecalis* is highly resistant to drugs inside the root canal system and is one of the micro-organisms that is resistant to the antimicrobial properties of calcium hydroxide (14).

Sodium hypochlorite (NaOCL) is a common substance used for canal cleaning and its antibacterial properties are due to the presence of hypochlorous acid. This substance is always considered in the field of washing and cleaning canals due to its high level of antibacterial activity and its ability in solving pulp tissue. But it emits a sharp smell and bothers patients while breathing, and at high concentrations has a toxic effect on periapical tissue and causes acute inflammation in the area (15, 16). On the other hand, it is very short-lived and has little lasting influence in the canal, and is associated with other adverse effects such as kerogen and discoloration of dental instruments (17). Therefore the use of a variety of plant irrigants is highly desirable, because in addition to having antiseptic properties, they are well accepted by patients.

Green tea consists of plant compounds that have antibacterial properties against many micro-organisms of the oral cavity. The scientific name of green tea is *Camellia sinensis* and is a member of the Theaceae family (18). Green tea powder is made during the drying and crushing of fresh leaves of the plant and is produced without passing through the stage of oxidation and fermentation. Phenolic compounds in extracts of green tea are effective in inhibiting the growth of decay-causing bacteria (19). In recent studies, researchers have found that green tea polyphenols reduce teeth decay by inhibiting oral streptococci (20, 21). Rezaei-Soufi et al. showed that the combination of green tea extract polyphenols as a natural compound has an antimicrobial effect, but the effect of chlorhexidine and fluoride is more than that of green tea and normal saline (22). Hashemi et al. found that green tea extracts, in comparison with antibiotics such as cefotaxime and ceftazidime, have more effective antibacterial effects against *Pseudomonas aeruginosa* that contain lactamase (23). Pujar et al. investigated the antimicrobial impact of triphala, green tea, and sodium hypochlorite 3% on *E. faecalis* bacteria. These researchers reported that due to the adverse effects of sodium hypochlorite, perhaps these plants can be used as root canal irrigants (24).

2. Objectives

This study aimed to compare the antimicrobial effects of different concentrations of green tea and 1% sodium hypochlorite on *E. faecalis* (most common isolated microorganisms from the failed canals) in a laboratory setting to find a more appropriate alternative for sodium hypochlorite

3. Materials and Methods

It was an experimental study which was conducted using the disk diffusion method. Green tea (Timen Co., Lahijan, Iran) was extracted with hot water. Aqueous extracts were then filtered, concentrated, and extracted twice with chloroform (Sigma-Aldrich Co., Germany). The remaining aqueous phase was extracted again with ethyl acetate (Sigma-Aldrich Co., Germany) to remove residual chloroform (Sigma-Aldrich Co., Germany). The extracts were then air-dried under vacuum, dissolved in ethanol (Razi Co., Ahwaz, Iran), and filtered. The final extract was obtained after ethanol was evaporated under vacuum. This extract, which consists primarily of tea pigments, has a dark brown color and is readily dissolved in water.

In this experiment, filter paper discs (Padtan Teb Co., Tehran, Iran) were used, which were impregnated with a certain amount of green tea extract with concentrations of 40, 80, 100 and 200 mg per mL and 1% sodium hypochlorite (Kimia Co., Tehran, Iran).

E. faecalis ATCC 29212 was used as a challenging microorganism; it was prepared from the regional center for persian type culture collection, Tehran, Iran. A 0.5 McFarland suspension of the *E. faecalis* was prepared in Tryptic soy broth (Merck, KGaA, Darmstadt, Germany) culture media and inoculated into the prepared culture media.

Then, a Mueller-Hinton agar culture medium (Merck, KGaA, Darmstadt, Germany) was used for isolated colonies and incubated at 37°C for 24 - 48 hours. The colonies formed were again transferred into Salin and incubated at 37°C under aerobic conditions for 24 hours. Subsequently, spectrophotometry was used to prepare a standard suspension of *E. faecalis* in the Saling 1.5×10^8 (CFU/mL) bacteria in each mm equivalent to 0.5 McFarland standard. The suspension was homogeneously spared onto the surface of the Mueller-Hinton agar by using a sterile cotton swab. Culture plates were left motionless for 2 to 5 minutes to absorb moisture. The paper disks with concentrations of 40, 80, 100 and 200 mg per mL of green tea extract were used to measure the effect of higher concentrations. Tetracycline (Padtan Teb Co., Tehran, Iran) was used as a positive control and distilled water as a negative control. After incubation for 24 - 48 hours at 37°C, the of inhibition zone was measured in millimeters by Caliper (Mitutoyo Co., Kyoto, Japan) with an accuracy of a tenth of a millimeter and the results were registered in the checklist. To increase the level of accuracy, each of which was cultivated 10 times. Finally the average diameter of inhibition zones was compared in the different groups.

The collected data was analyzed using SPSS software version 18. To compare the average diameter of the inhibition of bacterial growth caused by the antibacterial effects of different concentrations of green tea extract and hypochlorite, the ANOVA test was used and the Tukey statistical test was used to determine the difference between the mean diameters of the inhibition of bacterial growth in different concentrations of groups under study. The significant level in tests was considered 0.50.

4. Results

In this study, the inhibition zone of *E. faecalis* was studied in contact with different concentrations of green tea (40, 80, 100 and 200 mg per mL) and 1% sodium hypochlorite, with tetracycline serving as a positive control and distilled water as a negative control. The mean ± SD of the diameter of the inhibition zone of green tea with a concentration of 40 mg per mL was significantly lower compared to the mean ± SD of the inhibition zone of sodium hypochlorite 1% ($P < 0.001$). This result was repeated in other concentrations of green tea (80, 100 and 200 mg per milliliter). This means that in terms of statistics, hypochlorite 1% was significantly more effective compared to the concentrations used in green tea extracts in inhibiting the growth of *E. faecalis* ($P < 0.001$) (Table 1).

Table 1. Comparison of the Mean of Inhibition Zone Diameter (mm) of Different Concentrations of Green Tea and 1% Sodium Hypochlorite Against *Enterococcus faecalis*

	Minimum	Maximum	Mean ± SD
Tea Extract Concentration, mg/mL			
40	6.6	7.7	7.22 ± 0.37
80	8.7	9.6	9.07 ± 0.29
100	10	10.8	10.36 ± 0.28
200	13.6	14.5	14.5 ± 0.28
1% Sodium Hypochlorite			
40	18.6	20	19.13 ± 0.52
80	18.2	20	19.17 ± 0.63
100	18	20	18.93 ± 0.71
200	18.1	20	18.95 ± 0.65
P Value	< 0.0001	< 0.0001	< 0.0001

The results showed that in the plates containing green tea with a concentration of 40, 80, and 100 mg per mL, the mean ± SD of the diameter of the inhibition zone of tetracycline (positive control) equals 23.49 ± 0.27 mm, 23.56 ± 0.25 mm, and 23.52 ± 0.26 mm respectively, and in the plate containing green tea with a concentration of 200 mg per mL, the mean ± SD of the diameter of the inhibition zone of tetracycline (positive control) was 23.25 ± 0.27 mm. The Kolmogorov-Smironov test showed that the diameter of the inhibition zone data for different concentrations of green tea using the had a normal distribution ($P > 0.05$) (Table 2).

Table 2. Evaluation of the Normal Distribution Data of the Diameter of the Inhibition Zone of Different Concentrations of Green tea Against *Enterococcus faecalis*

Tea Extract Concentration, mg/mL	Kolmogorov-Smironov	P Value	Result
40	0.466	0.982	Normal distribution
80	0.503	0.962	Normal distribution
100	0.589	0.879	Normal distribution
200	0.537	0.935	Normal distribution

The results also showed that the mean diameter of the inhibition zone of different concentrations of green tea against Enterococcus bacteria had significant statistical differences ($P < 0.001$) (Table 3). Paired comparison tests also showed that the mean diameter of the inhibition zone of the different concentrations of green tea against *E. faecalis* had significant statistical differences ($P < 0.001$), so that the effect of 200 mg per mL was more than other concentrations of the extract (Table 3). By using Levene's test, the inhibition zone diameter variance of different concentrations of green tea was studied and no significant statistical difference was observed ($P = 0.679$).

5. Discussion

To clean the root canal system, a variety of irrigants are used. In general, a substance is more appropriate if in addition to greater antimicrobial efficacy, it has fewer adverse effects. To this end, researchers in recent years have tried different substances to achieve better results. Sodium hypochlorite at a concentration of 1% is used as a standard in the cleaning of the root canal due to its widespread usage (25). The present study showed that although all four concentrations of green tea hydroalcoholic extract have antibacterial effects and prevent the growth of *E. faecalis* and this effect significantly increased with an increase in extract concentration, the effect of sodium hypochlorite 1% was significantly higher in comparison with all concentration levels used in green tea. Similar to the results

Table 3. Comparison of the Mean Inhibition Zone Diameter (mm) of Different Concentrations of Green Tea Against *Enterococcus faecalis* (N = 10)

Tea Extract concentration, mg/mL	Minimum	Maximum	Mean ± SD	P Value
40	6.6	7.7	7.22 ± 0.37	< 0.001
80	8.7	9.6	9.07 ± 0.29	< 0.001
100	10.0	10.8	10.36 ± 0.28	< 0.001
200	13.6	14.5	14.05 ± 0.28	< 0.001

of this study, Prabhakar et al. (9) also showed that the effect of sodium hypochlorite on *E. faecalis* was stronger than that of green tea. But sodium hypochlorite 5% was used in their study, and the concentration of green tea used in the study was a single dose (60 mg/mL). In their study, the diameter of the inhibition zone in a plate containing sodium hypochlorite 5% was approximately two times larger than in our study. However, because in our study sodium hypochlorite with lower concentration can also inhibit these micro-organisms, due to the unpleasant side effects of this substance, there is no need to raise the concentration so much. Moreover, the method of comparison between the numbers of bacteria was used in their study, but in the recent study, the effect of these substances was measured on the basis of the difference between the diameters of inhibition zones.

Moghbel et al. investigated the antibacterial effect of green tea at concentrations of 1%, 0.2% and 0.5% compared with chlorhexidine 0.2%. According to the obtained results, through an increase in the concentration of green tea, the effect of this extract on *Streptococcus mutans* and *Staphylococcus aureus* increases, so that 1% concentration of tannin in green tea extract with chlorhexidine has a similar effect in the reduction of mouth aerobic bacteria. Therefore, instead of using chemical mouthwashes such as chlorhexidine, this herbal mouthwash is recommended especially for children and pregnant women. Although the bacterial strains used in their study are not identical to this one, in both studies, with an increase in concentration of green tea extract, their antimicrobial activity is also increased (26). Neyestani et al. investigated the effect of green tea in comparison with black tea on *Streptococcus* pyogenes strains; they also stated that an increase in the concentration of green tea extract increases its effect, although in their research the studied pathogens were different from this study. Also according to their results, the effect of green tea in similar concentrations was more than that of black tea on micro-organisms (27), which is probably due to the changes that occur after oxidation of green tea leaves.

Noormandi and Dabaghzadeh also showed that not only does antimicrobial activity of the compound increase by increasing the concentration of green tea, but also that at the same concentrations with an increase in the amount of the substance, antimicrobial activity of the compound increases as well. Their results indicated that although green tea in comparison with common antibiotics does not have a significant effect on *streptococcus*, it is definitely successful in strengthening the effect of this pharmaceutical group (28).

Pujar et al. investigated the impact of antimicrobial Triphala, green tea polyphenols, and sodium hypochlorite 3% on *E. faecalis* (24). Their results showed that sodium hypochlorite had the most antimicrobial effect on this bacterium and could destroy it completely, but green tea polyphenols and Triphala also showed significant antibacterial activity. These researchers reported that due to the adverse effects of sodium hypochlorite, these plants can be used as irrigants of the root canal (25). Kamath et al. studied the antimicrobial activity of green tea oil, 3% sodium hypochlorite, and 2% chlorhexidine on *E. faecalis*. The results of this study, unlike our study, showed that green tea oil, almost as much as sodium hypochlorite but less than chlorhexidine, has a significant antimicrobial effect on this pathogen (29). Therefore, the produced therapeutic composition of this substance may be able to change its effect on different pathogens such as *E. faecalis*. It is noteworthy that in all studies, the effect of green tea extract is compared with other common ingredients, and it seems that it is not easy to give an opinion on its absolute effect. In addition, perhaps by using different laboratory methods that could not be applied because of restrictions in the center, different results would be obtained.

5.1. Conclusions

The results of this study showed that green tea extract was able to create a zone of inhibition around *E. faecalis*. But the effect of sodium hypochlorite 1% on growth inhibition of *E. faecalis* was significantly higher than the green tea concentrations used in this study, and the use of higher concentrations in future studies is recommended.

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Footnotes

Authors' Contribution: Study concept and design: Farimah Sardari and Samira Hajisadeghi; analysis and interpretation of data: Farimah Sardari and Samira Hajisadeghi; acquisition of data: Farimah Sardari and Samira Hajisadeghi; drafting of the manuscript: Farimah Sardari.

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