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Antibacterial effects of calcium hydroxide and chlorhexidine gluconate

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ABSTRACT

The aim of this study is the comparison of antibacterial effects of calcium hydroxide medicament and chlorhexidine gluconate gel on *Enterococcus faecalis.*

The study is composed of three experimental groups. Antibacterial effects of group 1, calcium hydroxide medicament (Calcicure); group 2, %1 chlorexidine gluconate gel (Corsodyl) and group 3, combination of calcium hydroxide medicament plus chlorexidine gluconate gel equal ratio were examined with agar diffusion method on the 1st, 2nd and 7th days. Inventions are evaluationed as statistical with ANOVA and multiple comparisons tests.

In the end of our study for the three time period the antibacterial effect of chlorexidine gluconate gel was more than calcium hydroxide medicament and the combination of calcium hydroxide medicament plus chlorexidine gluconate gel equal ratio (P<0,01). After the 1st and 2nd days' measures between the antibacterial effects of calcium hydroxide medicament and combination of calcium hydroxide medicament + chlorhexidine gluconate gel on this bacteria there was no important statistical difference (P>0,05), however on the 7th day it is found an important statistical difference (P<0,01).

The all experiment materials that is used showed an increasing antibacterial effect on *E.faecalis.*

Keywords: Calcium hydroxide medicament, Chlorhexidine gluconate gel, *Enterococcus faecalis,* Antibacterial effect

ÖZET

Kalsiyum hidroksit içerikli medikaman ve klorheksidin glukonat jel ve her iki medikamanın eşit oranda karışımının *Enterococcus faecalis* üzerindeki antibakteriyel etkilerinin karşılaştırılması amaçlanmıştır.

Çalışmada 3 deneysel grup oluşturuldu , I. grupta kalsiyum hidroksit içerikli medikamanın (Calcicure), II. grupta %1'lik klorheksidin glukonat jelin (Corsodyl), III. grupta eşit oranda hazırlanmış kalsiyum hidroksit içerikli medikaman + klorheksidin glukonat jel karışımının antibakteriyel etkileri 1. 2. ve 7. günlerde agar difüzyon yöntemiyle incelendi. Bulgular ANOVA ve Çoklu karşılaştırma testleri ile istatistiksel olarak değerlendirildi.

Çalışmamız sonucunda ölçüm yapılan üç zaman periyodunda da klorheksidin glukonat jel; kalsiyum hidroksit içerikli medikaman ve kalsiyum hidroksit içerikli medikaman + klorheksidin glukonat jel karışımından daha fazla antibakteryel etki gösterdi. (P<0,01) Kalsiyum hidroksit içerikli medikaman ve kalsiyum hidroksit içerikli medikaman + klorheksidin glukonat jel karışımının bu bakteri üzerindeki antibakteriyel etkileri arasında ise 1. ve 2. gün ölçümlerinde istatistiksel olarak anlamlı bir fark bulunmazken (P>0,05), 7. gün yapılan ölçümlerde istatistiksel olarak anlamlı fark bulundu (P<0,01).

Kullanılan tüm deney materyalleri E. Faecalis'e karşı zaman geçtikçe artan antibakteriyel etki gösterdiler.

Anahtar Kelimeler: Kalsiyum hidroksit içerikli medikaman, Klorheksidin glukonat jel, *Enterococcus faecalis*, Antibakteriyel etki

Introduction

The root canal system has a complex structure in which microorganisms can harbor, such as dentinal tubules, accessory canals, canal ramifications, apical deltas and transverse anastomoses.[1] Successful endodontic treatment is primarily aimed at removing microorganisms from these difficult areas of the pulp cavity. In addition to mechanical preparation and irrigation, it is recommended that intracanal medication be used when necessary.[1,2,3] When selecting intracanal medication, it is important to be aware of the types of microorganisms present in the root canal, the mechanism of action of the medication, how long it remains in the canal, its diffusion properties, its toxicity and the inflammatory reactions it causes in the area of application.[4,5]

The role of bacteria in the development of pulpal and periapical diseases has been proven in many studies.[6,7] The presence of bacteria leads to pulpal or periapical inflammatory reaction.[8] The severity of the inflammatory response of pulpal and periapical tissue is related to the presence, number and contact time of microorganisms.[9]

Single-session root canal treatment is indicated for teeth with vital pulp, whereas endodontic treatment of a tooth with an infected necrotic pulp should be performed in at least two sessions. The aim of this multi-session treatment is to place an antimicrobial agent in the root canal to ensure that the remaining bacteria are destroyed between sessions.[10]

The purposes of using in-canal medication in the root canal treatment of teeth with necrotic pulp or periapical lesions can be summarised as follows: To eliminate bacteria remaining in the root canal after biomechanical expansion, to reduce inflammation of the periapical tissue and pulp residues left behind, to render the substances remaining in the canal harmless and neutralise tissue residues, to create a barrier against leakage from the temporary filling material, to dry canals with continuous exudate.[1,11,12]

In today's endodontic practice, calcium hydroxide-containing media are generally used to achieve the above-mentioned objectives. However, since the antibacterial effect of calcium hydroxide against stubborn pathogens is limited, the development of alternative or antibacterial agents that can be used in combination with calcium hydroxide is ongoing.[13] Chlorhexidine gluconate is one of them.

Chlorhexidine gluconate has a bactericidal effect after a prolonged bacteriostatic effect. Chlorhexidine is effective against most microorganisms such as gram (+), gram (-) bacteria, yeast and some fungi and viruses. Chlorhexidine delays bacterial growth. It is absorbed from microbial cell walls and causes disruption of membrane integrity.[14]

Chlorhexidine gluconate inhibits cell membrane enzymes at low concentrations and increases the permeability of the cell membrane. This effect is called bacteriostasis. At high concentrations, it causes precipitation of cytoplasmic organelles and shows bactericidal effect. It has been reported that slow release of chlorhexidine gluconate plays a major role in its long-term antibacterial effect.[15]

Characteristics of *E.faecalis*

Enterococcus faecalis is a non-spore, fermentative, facultative gram (+) anaerobic cocci that can be classified as an opportunistic pathogen. Its cells are ovoid shaped. They are usually observed singly, in pairs or in short chains. They are nonhaemolytic and immobilised bacteria.[16] They can lower the pH of the medium up to 4.1-4.6. They can survive for 15 minutes at 60 °C. Their main reservoirs are faeces and mouth. They may cause subacute endocarditis and urinary system infection.[17]

It has been observed that facultative bacteria usually dominate the environment in unsuccessful root canal treatments. It has been reported that *Enterococcus faecalis* is one of the most frequently isolated bacteria from the root canal, but Enterococcus faecalis is not frequently encountered in primary infections in the root canal.[10] This suggests that Enterococcus faecalis is transmitted to the root canal during endodontic treatment. It has been reported that bacteria can enter the canal during root canal treatment, between sessions or when the root canal remains open.[18]

Enterococcus faecalis can lower the pH of the environment to 4.1-4.6. This bacterium has been shown to be resistant to various medications. If it has settled in the root canal, it is very difficult to eliminate it by conventional methods.[19] It has mechanisms that enable it to survive in an unfavourable environment: Survival

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without nutrients, survival in the presence of intra-canal drugs and irrigants, survival in high salinity, antibiotic resistance, invasion of dentin tubules, using fluids from the periodontal ligament as food are among the main ones.[20]

Enterococcus faecalis biofilm consists of exopolysaccharides, proteins, lipids and extracellular deoxyribonucleic acid (eDNA).[21,22] The dense and protected environment of the biofilm facilitates gene transfer and increases the stability of the biofilm. In Enterococcus faecalis biofilm, eDNA is actively released from autolysis of the bacterium itself or its mimic or from membrane vesicles and nanofibres [21,23]

The presence of extracellular polymer matrix around the biofilm makes it more resistant to conventional root canal treatment. Therefore, the use of intra-canal medications such as chlorhexidine, tetracycline, calcium hydroxide is recommended.[24,25]

In our study, we aimed to investigate the antibacterial effects of calcium hydroxide, chlorhexidine gluconate gel and the mixture of these two agents on *Enterococcus faecalis* in-vitro by agar diffusion method on days 1, 2 and 7.

Materials and Methods

Antibacterial properties of calcium hydroxide containing medikaman (Calcicure-voco Germany), chlorhexidine gluconate gel (Corsodyl-gsk Ireland) and calcium hydroxide containing medikaman + chlorhexidine gluconate gel mixture were investigated by agar diffusion method.

The bacterial strain to be used in our study was prepared according to Mc-Farland 0.5 standard in the Microbiology Laboratory of Ankara University Faculty of Dentistry Microbiology Laboratory and was inoculated into the broth and kept in an oven at 37°C for 24 hours.

In 6 petri dishes, blood agar medium infected with bacteria was used. Each petri dish was divided into three sections and three standard blanks were made in each section. Then, they were swabbed with sterile swabs on 7% sheep blood Müller-Hinton agar medium with standard slots of 2 mm depth and 4.5 mm width.

The media prepared in accordance with the manufacturer's recommendations were injected into the prepared nests in equal amounts with the help of macropipettes.

The prepared medium was evaluated in terms of the antibacterial effects of the materials at three different time periods, at the end of 24 hours, at the end of the second day and at the end of the seventh day. These evaluations were performed by measuring the length of the inhibition zone around the samples in the petri dishes millimetrically with caliper measuring instrument. Average values were taken for irregular zones around the samples.

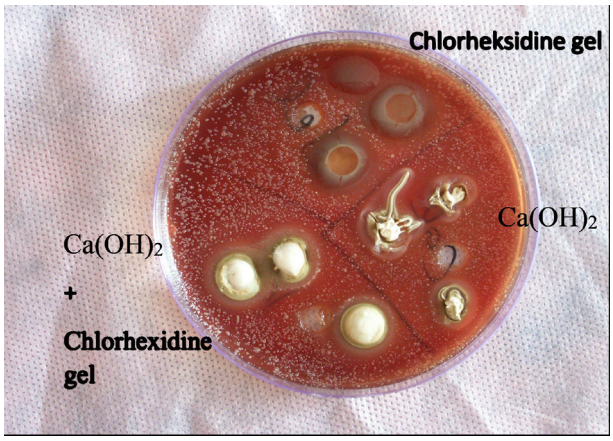

Figure 1. One of the 6 petri dishes used in the study shows inhibition areas (white areas around the standard blanks) formed at the end of day 1. Calcium hydroxide is abbreviated as $Ca(OH)_2$.

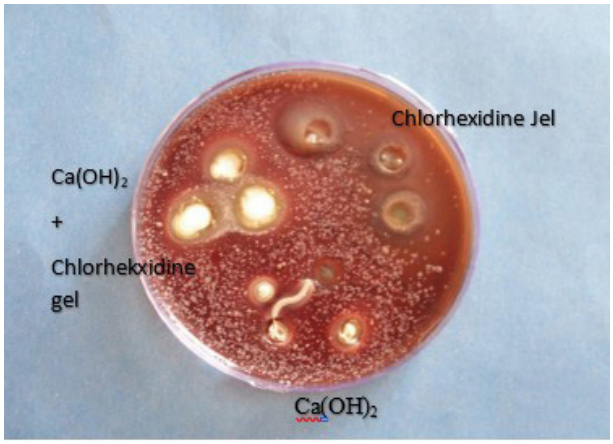

Figure 2. Inhibition areas formed at the end of day 2

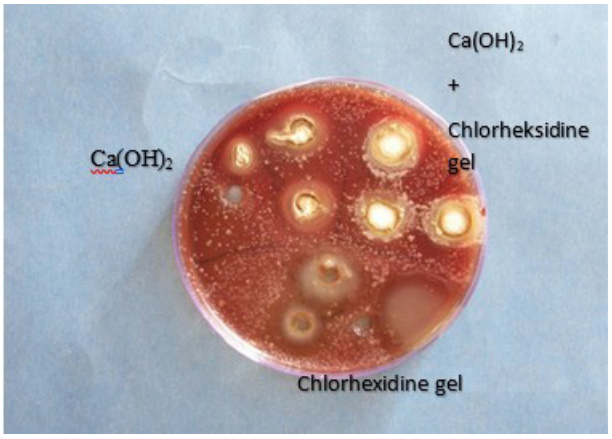

Figure 3. Inhibition areas formed at the end of the 7th day

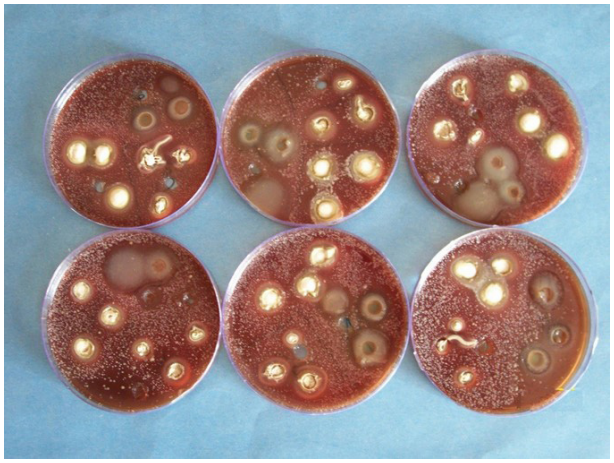

Figure 4. Images of 6 petri dishes used in the study at the end of the 7th day

Statistical Analysis

ANOVA test and multiple comparison test were used statistically in our study. For each of the three time periods, the millimetre lengths of the inhibition areas formed by each material were averaged. One-way ANOVA test was performed to determine whether there was a significant difference between the antibacterial effects of the materials as of the experimental days. P value less than 0.05 was accepted for significance.

Results

The diameters of the inhibition zones formed as a result of the study were measured in millimetres on the 1st, 2nd and 7th days. Calcium hydroxide was determined as group 1, chlorhexidine gluconate as group 2 and the mixture as group 3. The measurement results are given in the following tables:

Table I. The diameters of the inhibition zones formed by the experimental materials at the end of the first 24 hours

Millimeter	Calcium Hydroxide (Group 1)			Chlorhexidine Gluconate (Group 2)			Calcium Hydroxide + Chlorhexidine Gluconate (Group 3)		
I. petri dish	12	9	11	19	16	15	10	11	10
II. petri dish	8	8	10	16	19	22	9	12	9
III. petri dish	6	8	7	22	20	24	10	9	9
IV. petri dish	4	5	6	15	19	19	6	8	9
V. petri dish	3	3	6	16	15	13	6	10	8
VI. petri dish	6	8	5	13	14	15	8	7	6

Table II. The diameters of the inhibition zones formed by the experimental materials at the end of the 2nd day

Millimeter	Calcium Hydroxide (Group 1)			Chlorhexidine Gluconate (Group 2)			Calcium Hydroxide + Chlorhexidine Gluconate (Group 3)		
I. petri dish	13	10	11	26	25	23	11	13	12
II. petri dish	8	9	11	18	18	23	11	13	10
III. petri dish	7	9	9	25	21	24	10	9	10
IV. petri dish	3	6	7	17	20	19	7	8	9
V. petri dish	3	3	7	17	17	15	7	11	9
VI. petri dish	7	8	5	14	15	16	10	7	6

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Table III. The diameters of the inhibition zones formed by the experimental materials at the end of the 7th day

millimeter	Calcium Hydroxide (Group 1)			Chlorhexidine Gluconate (Group 2)			Calcium Hydroxide + Chlorhexidine Gluconate (Group 3)		
I.petri dish	16	14	14	32	30	28	16	18	17
II.petri dish	11	13	15	24	26	29	16	18	15
III.petri dish	10	12	13	32	29	30	14	14	16
IV.petri dish	6	10	12	22	24	25	12	14	15
V.petri dish	6	5	11	22	23	24	12	14	15
VI.petri dish	10	10	12	20	22	24	15	13	12

For each of the three time periods, the average of the millimetre lengths of the inhibition areas formed by each material was taken. One-way ANOVA test was performed to determine whether there was a significant difference between the antibacterial effects of the materials on the measured days. A p value less than 0.05 was accepted for significance.

According to the results of one-way ANOVA test, there was a statistically significant difference (P<0.05) between the calcium hydroxide-containing medikaman, chlorhexidine gluconate gel and mixture groups of both in all three time periods (1st, 2nd and 7th day measurements) (Tables 5-6-7).

Table IV. ANOVA test for first day measurements

Variance source	Degrees of freedom	Sum of squares	F value	P value
Between Groups	2	1159,259	64,170	P <0,05
Within groups	51	460,667		
General	53	1619,926		

Table V. ANOVA test for day 2 measurements

Variance source	Degrees of freedom	Sum of squares	F value	P value
Between Groups	2	1517,481	80,588	P <0,05
Within groups	51	480,167		
General	53	1997,648		

Table VI. ANOVA test for day 7 measurements

Variance source	Degrees of freedom	Sum of squares	F value	P value
Between Groups	2	2131,704	119,557	P <0,05
Within groups	51	454,667		
General	53	2586,370		

The materials were compared with each other for three time periods based on the average of the millimetric diameters of the inhibition areas they created in the regions where they were applied. Multiple comparison tests were used for this purpose.

According to multiple comparison tests, inhibition areas were found to be higher in Group2 on the 1st day compared to the other groups and this result was found to be statistically significant (p<0.05). There was no statistically significant difference between Group1 and Group3.

Table VII. Comparison of 3 groups with each other in the first day measurements (Between Group 1 and Group 2 : There is a difference (P<0,05) Group 2 > Group 1 , Between Group 1 and Group 3: No difference (P>0,05), Between Group 2 and Group 3 : There is a difference (P<0,05) Group 2> Group 3)

Day 1	Group 2	Group3
Grup 1	There is a difference (P<0,05) Group 2 > Group 1	No difference (P>0,05)
Group 2	----------	There is a difference (P<0,05) Group 2> Group 3

At the end of the 2nd day, there was no statistically significant difference between Group 1 and Group 3. Group 2 was found to be statistically significantly different from the other groups (P<0.05).

Table VIII. Comparison of 3 groups with each other in the second day measurements. Between Group 1 and Group 2 : There is a difference (P<0,05) Group 2 > Group 1, Between Group 1 and Group 3: No difference (P>0,05), Between Group 2 and Group 3 : There is a difference (P<0,05) Group 2> Group 3

Day 1	Group 2	Group3
Grup 1	There is a difference (P<0,05) Group 2 > Group 1	No difference (P>0,05)
Group 2	----------	There is a difference (P<0,05) Group 2> Group 3

At the end of the 7th day, the difference between Group 1 and Group 2 was found to be statistically significant, as in the other days. In addition, the difference between Group 1 and Group 3 was found to be statistically significant, unlike the results of the measurements made on the other days. Again, a statistically significant difference was found between Group 2 and Group 3 on the 7th day, as in the measurements made on the other days (P<0.05).

It was observed that the diameters of the inhibition areas increased with time in all three groups.

The materials were statistically evaluated in terms of the increase in their effects on *Enterococcus faecalis* on days 1, 2 and 7. Repeated measures analysis of variance (ANOVA) was used for this evaluation and it was determined that the difference between the groups was statistically significant (P<0.05).

Table IX. Comparison of 3 groups with each other in the seventh day measurements. Between Group 1 and Group 2 : There is a difference (P<0,05) Group 2 > Group 1, Between Group 1 and Group 3:There is a difference (P<0,05) Group 3 > Group 1, Between Group 2 and Group 3 : There is a difference (P<0,05) Group 2> Group 3

Day 1	Group 2	Group3
Grup 1	There is a difference (P<0,05) Group 2 > Group 1	There is a difference (P<0,05) Group 3 > Group 1
Group 2	----------	There is a difference (P<0,05) Group 2> Group 3

Multiple Comparison Tests were applied to determine which time periods were statistically significant.

It was determined that the antibacterial effect of calcium hydroxide-containing medication on *Enterococcus faecalis* increased on the 2nd and 7th days compared to the 1st day and this increase was statistically significant (P<0.05).

It was determined that the antibacterial effect of chlorhexidine gluconate gel on E*nterococcus faecalis* increased on the 2nd and 7th days compared to the 1st day and this increase was statistically significant (P<0.05).

Similarly, it was determined that the increase in the antibacterial effect of calcium hydroxide + chlorhexidine gluconate gel mixture on *Enterococcus faecalis* on the 2nd and 7th days compared to the 1st day was statistically significant (P<0.05).

It was observed that the inhibition areas increased as time progressed in all 3 experimental groups. This can be interpreted as an increase in antibacterial effect as time progresses.

In Group 2, i.e. chlorhexidine gluconate group, inhibition areas were found to be significantly higher than the other groups in all 3 days.

Discussion

Calcium hydroxide medicaments have been used as in-canal medicaments in root canal treatment for a long time due to their positive properties such as antibacterial effect, stimulating hard tissue production and having alkaline pH. Studies have shown that calcium hydroxide medicaments placed in root canals can diffuse through dentinal tubules and foramen apicale.[26,27,28,29]

There are different recommendations regarding the duration of leaving calcium hydroxide-containing media in the canal. Byström et al. reported that all microorganisms were eliminated after leaving calcium hydroxide-containing media in the canal for 4 weeks.30 Reiht and Dahlen reported that infection persisted in 26% of the canals two weeks after calcium hydroxide application.[31] Sjögren et al. reported that bacteria were effectively eliminated in root canals treated with calcium

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hydroxide for one week.[32] Orstavik et al. detected bacteria in 34.8% of root canals after a one-week application of calcium hydroxide-containing media.[8] In their in vivo study, Barbosa et al. reported that positive cultures were obtained in 12 (26.7%) of 45 cases in which calcium hydroxide-containing medicaid was applied for one week.[33]

The widespread opinion about the time that calcium hydroxide should be left in the root canal system is at least one week. In our study, we planned the 7th day measurements considering this situation. We observed that the antibacterial effect increased in the 7th day measurements compared to the other measurements. As the antibacterial effect of calcium hydroxide is limited, we wanted to observe whether the antibacterial effect increases when mixed with chlorhexidine gluconate.

Chlorhexidine, which is known to have antibacterial effect as an irrigation solution, has been investigated in various studies in terms of its antibacterial activity when used as an intracanal medication. Heling et al. compared the effect of chlorhexidine gluconate and calcium hydroxide in the form of medikaman applied with a controlled release apparatus on bovine dentinal tubules infected with Enterococcus faecalis and observed a significant decrease in the bacterial population in samples in which chlorhexidine was used, whereas no such effect was detected in calcium hydroxide.[34] Siqueira and Uzeda reported that 0.12% chlorhexidine gluconate gel was more effective than calcium hydroxide in the form of medikaman in an in-vitro study in which they compared the antibacterial effects of different intra-canal medikamans.[35] These results are in parallel with the results of our study.

Enterecoccus faecalis has been reported to play an important role in resistant apical periodontitis cases requiring retreatment.[36] Because this microorganism is more resistant to locally used disinfectants than other microorganisms of the endodontic flora.[37,38] Considering the presence of *E.faecalis* in retreatment cases, chlorhexidine solutions are reported to be more effective.[38]

In the in-vitro studies of Almyroudy et al. where they examined the antibacterial effects of calcium hydroxide, chlorhexidine gluconate gel and a mixture prepared in equal proportions on *E.faecalis* on extracted human teeth on the 3rd and 8th days, the antibacterial effect of calcium hydroxide was observed on the 8th day compared to the 3rd day.[39] They found that the antibacterial effect of 1% chlorhexidine gluconate gel also increased over time. Researchers found that the difference between the effect of calcium hydroxide and the antibacterial effect of chlorhexidine gluconate on the 3rd and 8th days was statistically significant and that the effect of chlorhexidine gluconate was greater. This finding is also similar to our study.

They found a statistically significant difference between the antibacterial effects of calcium hydroxide and calcium hydroxide + chlorhexidine gluconate gel mixture in both time periods and reported parallel results with our findings. The researchers found no statistically significant difference between the antibacterial effects of chlorhexidine gluconate gel and calcium hydroxide + chlorhexidine gluconate gel mixture. Unlike the researchers, in our study, according to the measurements made on the 1st, 2nd and 7th days, chlorhexidine gluconate gel showed a statistically significant difference from the other experimental materials at all time periods. This difference may be attributed to the different materials and methods used.

Lin et al. evaluated the antibacterial effects of calcium hydroxide, 0.12% chlorhexidine gluconate gel and its mixture in equal proportions on E.faecalis in blood agar medium by agar diffusion method on days 1 and 3.[40] As a result of their study, they found that there was no statistically significant difference between the size of the inhibition areas formed by calcium hydroxide, chlorhexidine gluconate gel and the mixture of both on day 1. However, in our study, the antibacterial effect of chlorhexidine gluconate gel was statistically significantly different from the antibacterial effects of calcium hydroxide and calcium hydroxide + chlorhexidine gluconate gel mixture on day 1. This may be due to the low concentration of chlorhexidine gluconate gel used in that study. On the 3rd day, they found that chlorhexidine gluconate gel showed more antibacterial effect than the other experimental groups according to the mean size of inhibition areas and this difference was statistically significant. This finding is in parallel with our findings

on the 2nd and 7th days. Calcium hydroxide, on the other hand, showed less antibacterial effect than the other two groups on the 1st and 3rd days and reported results in line with our findings. They found no statistically significant difference between the antibacterial effects of chlorhexidine gluconate gel and calcium hydroxide + chlorhexidine gluconate gel mixture on days 1 and 3. Unlike Lin et al. study, in our study, a statistically significant difference was found between the antibacterial effects of chlorhexidine gluconate gel and calcium hydroxide + chlorhexidine gluconate gel mixture.

Schafer and Bossmann examined the antibacterial effects of calcium hydroxide, 2% chlorhexidine gluconate solution and calcium hydroxide + chlorhexidine gluconate combination on *E.faecalis* for one week in their in-vitro study on 40 extracted human teeth in 2005.[41] At the end of the 7th day, they found that 2% chlorhexidine gluconate had more antibacterial effect than calcium hydroxide and calcium hydroxide + chlorhexidine gluconate combination and reported that the difference was statistically significant. Although chlorhexidine gluconate was used as a solution in this study, the finding obtained is in parallel with our findings on the 7th day.

A study was conducted in 2008 to evaluate the efficacy of chlorhexidine gluconate gel, calcium hydroxide and their combination with iodoform and zinc oxide powder as intracanal pastes against persistent microorganisms and to measure the pH changes caused by these pastes.[42] Antimicrobial activity was determined by agar diffusion method, which is the same method as in our study. Growth inhibition zones were measured immediately after the preparation of the pastes, after 24 hours and after 1 week. The largest average microbial inhibition zones were produced by 2% CHX gel, followed by $Ca(OH)_2$ + 2% CHX gel + iodoform, $Ca(OH)_2$ + 2% CHX gel, $Ca(OH)_2$ + 2% CHX gel + zinc oxide and $Ca(OH)_2$ + water. The mean pH value of all medications except CHX gel (pH=7.0) remained above 12.0 throughout the experiment. The results of this study showed that all medications had antimicrobial activity, but the most effective against the tested microorganisms was 2% CHX gel, followed by its combination with $Ca(OH)_2$ and iodoform. These results are in parallel with our study.

In a study on endo-periodontal lesions, chlorhexidine-loaded calcium hydroxide pastes were used as intracanal medication.[43] These were prepared and tested on pathogens present in both the root canal and periodontal pocket. The 0.5% and 1% chlorhexidine-loaded paste reduced Porphyromonas gingivalis growth and was effective in eliminating or inhibiting Enterococcus faecalis biofilm. Chlorhexidine -loaded medicaments resulted in transradicular diffusion of active ingredients out of the tooth through the apex and lateral dentinal tubules, as indicated by Chlorhexidine release after 7 days and changes in pH and calcium concentrations.The results suggest that the root canal may serve as a reservoir for periodontal drug delivery and CHX-based calcium hydroxide medicaments may be an aid for the control of infection and inflammation in endo-periodontal lesions. The importance of this study for our study is that the calcium hydroxide medication loaded with chlorexidine has been studied in vivo and has been successful against persistent pathogens in endoperio lesions.

Gomes et al. evaluated the efficacy of 2% chlorhexidine gluconate gel and calcium hydroxide ($Ca(OH)_2$) as intracanal medicaments against Enterococcus faecalis injected into bovine maxillary incisors.[44] Chlorhexidine gel alone completely inhibited the growth of E. faecalis after 1, 2, 7 and 15 days. Calcium hydroxide allowed microbial growth at all experimental times. The combination of chlorhexidine and $Ca(OH)_2$ was effective after 1 and 2 days and showed 100% antibacterial effect; however, its antibacterial activity decreased between 7 and 15 days. They reported that 2% chlorhexidine gel alone was more effective than calcium hydroxide against E. faecalis, but its antibacterial activity depended on how long it remained in the root canal. Although the study methods are different from our study, it supports our study in terms of the successful antibacterial effect of chlorhexidine gluconate against Enteroccus faecalis in experimental times.

Conclusion

As a result of our study, it was determined that chlorhexidine gluconate gel showed statistically more antibacterial effect than other experimental materials in all time periods. The use of chlorhexidine gluconate, which is frequently used as an irrigation solution in the clinic, as an intra-canal drug in gel form when necessary,

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or the production of a medicament containing its mixture with calcium hydroxide may increase the success rate in multi-session treatments and in stubborn infections. We believe that more in vivo and in-vitro studies should be done on this topic.

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