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In vitro study of the Ferric sulfate and Formocresol antibacterial effect on Oral bacteria

Abstract

Today, pulpotomy using ferric sulfate has become more popular because of the side effects of formocresol. This study was conducted to investigate the antibacterial effect of ferric sulfate and formocresol on *Streptococcus mutans*, *Lactobacillus acidophilus,* and *Enterococcus faecalis.*

The antibacterial effect of ferric sulfate and formocresol (two types) on oral bacteria was investigated using the disk agar diffusion method. We had four groups for each effective combination. However, each experiment was performed in triplicate, and chlorhexidine 0.2% was used as a control antimicrobial agent. After collecting data using (SPSS 26) software, it was analyzed using Kruskal-Wallis and Mann-Whitney tests at an error level less than and equal to 0.05.

The most antibacterial effect was related to formocresol. However, the diameter of the no-growth zone of *Streptococcus mutans*, *Lactobacillus acidophilus,* and *Enterococcus faecalis* under the influence of type 1-formocresol (nikDarman®,Made in Iran) was 70, 72, 50 mm, and in type 2 (MasterDent® Made in USA) it was 72, 76, and 54 mm. Also, ferric sulfate was less effective than chlorhexidine, while the diameter of the no-growth zone of *Streptococcus mutans, Lactobacillus acidophilus,* and *Enterococcus faecalis* under the influence of ferric sulfate(Astringedent_Ultradent®,Made in USA) was 12, 24, and 12 mm, respectively. And for 0.2% chlorhexidine(Irsha®,Made in IRAN), it was 22, 26, and 24 mm, respectively.

The antibacterial effect of formocresol was much higher than ferric sulfate and chlorhexidine on *streptococcus mutans*, *Lactobacillus acidophilus*, and *Enterococcus faecalis.*

Keywords: *Antibacterial effect, Ferric sulfate, formocresol, streptococcus mutans, Lactobacillus acidophilus, Enterococcus faecalis*

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Introduction

There are various bacteria in the oral cavity, some of which play a major role in maintaining health (normal flora) and some in causing oral and dental diseases (pathogens). Thus reducing and eliminating pathogenic bacteria is crucial in preventing dental caries and oral diseases, while maintaining the permitted range of normal oral flora. One of these pathogenic bacteria is oral streptococci, which are an important part of the dental plaque set and one of the most important members of this set is *streptococcus mutans*, which plays a major role in dental caries (1). Also, Enterococci are part of the mouth microbial flora and cause many of the primary root canal infections and have been isolated from a large number of root canals of treated teeth with chronic (failed) apical periodontitis. *Enterococcus faecalis* is a gram-positive facultative anaerobic bacterium causing oral and dental infections in humans (2). Also, *Streptococcus salivarius* with its presence and with a lower adhesive strength compared to *streptococcus mutans* can be involved in the formation of dental plaque (3). *Streptococcus sanguinis* is also present as the mouth's normal flora, but its excessive number causes the accumulation of other microbial factors and the formation of dental plaque (4). Also, lactobacilli such as *Lactobacillus casei* are related to the development of dental caries (5).

With increasing the number of antibiotic-resistant strains of these bacteria, many efforts have been made to introduce the best method to reduce the rate of bacteria and maintain oral and dental health (6). Pulpotomy is one of the most common treatments administrated for deciduous molar teeth exposed to caries. However, pulpotomy with formocresol has been used for many years as the gold standard method in the treatment of the pulp of deciduous molar teeth, and it is still welcomed by dentists due to its ease of application and high

clinical success (7). Due to its side effects, formocresol has lost its popularity in the last two decades, and researchers have been encouraged to find alternative methods (8, 9). Recently, ferric sulfate pulpotomy is one of the methods that has attracted the most attention and has been preferred over formocresol due to its lack of toxic and mutagenic properties as well as reducing the clinical work time (10).

Based on the researchers, formocresol and ferric sulfate have the same positive antibacterial effect (11). It has been reported that the antibacterial effect of ferric sulfate in laboratory conditions is similar to 0.2% chlorhexidine digluconate on oral microorganisms such as *Staphylococcus aureus, Enterococcus faecalis, Candida albicans, Porphyromonas gingivalis and Lactobacillus acidophilus* (12,13). Some studies have also reported that the clinical and radiographic success of formocresol pulpotomy is higher than ferric sulfate pulpotomy (14-17), depending on the fixing and disinfecting properties of formocresol. A study has reported that ferric sulfate only has hemostatic properties and does not have killing or inhibiting properties of bacteria (18). Ferric sulfate preserves maximum viable tissue without inducing restorative dentin. Although its mechanism has not been fully clarified, it is believed that iron ions and ferric sulfate chemically react with blood proteins and cause agglutination of these proteins, and the membrane of this complex mechanically covers the cut-off blood vessels and creates hemostasis and causes clot formation (18).

Many studies have examined the antibacterial properties of formocresol and ferric sulfate. Additionally, some studies have reported that iron ion is effective in preventing respiratory viruses such as Sever Acute Respiratory Syndrome (SARS) and Coronaviruses. However, conflicting results have been reported about the lethal effect of ferric sulfate on bacteria. Thus, given the importance of oral bacteria in pulpotomy treatment and other dental treatments, it is of particular importance to recommend the use of the best intra-canal drugs that also have antibacterial properties. Thus, the present study aimed to evaluate the antibacterial effect of ferric sulfate and formocresol on important oral bacteria including *streptococcus mutans, Lactobacillus acidophilus*, and *Enterococcus faecalis*.

Materials and Methods

The present in vitro study was conducted to investigate the antibacterial effect of ferric sulfate(Astringedent_Ultradent®,Made in USA) and formocresol (two types), type 1 (NickDarman®,Made in Iran), and type 2 (Master-Dent®,Made inUSA) on *S. mutans, L. acidophilus*, and *E. faecalis* by disk agar diffusion method. Accordingly, the standard strains of the desired bacteria were purchased from the Iranian Research Organization for Science and Technology (IROST) and were cultured on specific or public media (Mitis Salivarius agar (Sigma, Germany) for *S. mutans*, MRS agar (Condalab, Spain) for *L. acidophilus*, and blood agar (Condalab) for *E. faecalis*). Then, *S. mutans* and *E. faecalis* were incubated at 37°C under aerobic conditions and in the presence of 5% CO2, while *L. acidophilus* was incubated at 37°C under anaerobic conditions. After the growth of the bacteria at 48 hours, standard diagnostic tests were performed to identify the bacteria. Then, according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI), the disk agar diffusion test was performed (18).

In this test, the bacterial colonies were dissolved in 0.85% normal saline to prepare turbidity of the desired bacteria equivalent to 0.5 McFarland (1.5×10^8 cfu/ml). Then, the 0.5 McFarland suspension was inoculated on Mueller Hinton agar (Merck, Germany) using a sterile cotton swab in three different directions (18). Next, we placed 6 mm blank paper disks on the culture medium and inoculated 20 µl of different concentrations of the antimicrobial agents on each of the disks. Then, the plates were incubated for 48 hours at 37 °C under specific conditions for each bacterium. Then, the no-growth zone of bacteria was assessed by a ruler. The no-growth zone diameters were compared with the positive control (0.2% chlorhexidine (Irsha®,Made in IRAN) (18). After collecting data using (SPSS 26) software, results less than and equal to 0.05 were analyzed with Shapiro-Wilk tests to check normality, and significance was expressed using Kruskal-Wallis and Mann-Whitney Tests. This article was derived from the thesis with a code of ethics of IR.MAZUMS.REC.1401.14226 from Sari University of Dentistry (Mazandaran).

Results

The most antibacterial effect against the tested bacteria was related to formocresol type 2, followed by formocresol type 1. Also, ferric sulfate was less effective than Chlorhexidine (figure 1).

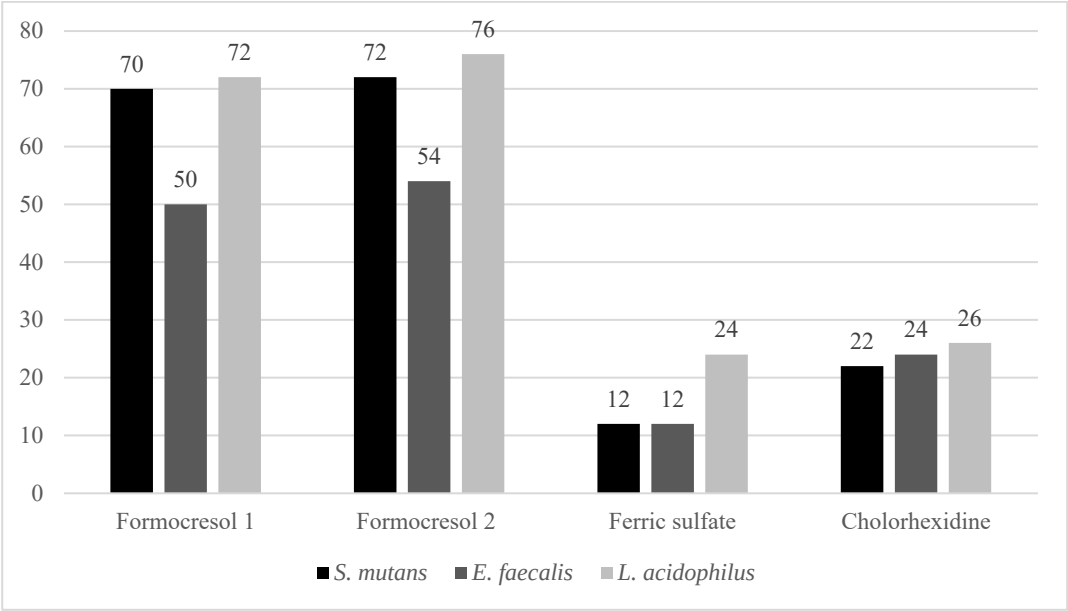

Figure 1. No-growth zone diameters (mm) of tested bacteria under the effect of tested antibacterial agents

The results showed that the no-growth zone diameter of *S. mutans*, *E. faecalis*, and *L. acidophilus* was different (Table 1).

Table 1. The antibacterial effect of the tested agents

bacteria	Kruskal Wallis statistic	p-*value*
Streptococcus mutans	9.74	0.021
Enterococcus faecalis	10.39	0.016
Lactobacillus acidophilus	9.66	0.022

The no-growth zone diameters of *E. faecalis* under the effect of

Formocresol 2 was significantly higher than Formocresol 1(p=0.05), but the no-growth zone

diameters of *S. mutans* and *L. acidophilus* were not significantly different (p<0.05). The no-

growth zone diameters of all three bacteria against formocresol type 1 were significantly

greater than the Ferric sulfate (p=0.05)

Also, the no-growth zone diameters of *S. mutans*, *E. faecalis*, and *L. acidophilus* against formocresol type 1 were significantly greater than Chlorhexidine (p=0.05). Moreover, the no-growth zone diameters of all three bacteria under the effect of formocresol type 2 were significantly greater than ferric sulfate (p=0.05).

On the other hand, the no-growth zone diameters of *S. mutans, E. faecalis*, and *L. acidophilus*

against formocresol type 2 were significantly greater than Chlorhexidine (p=0.05). Also, the

no-growth zone diameters of *S. mutans* and *E. faecalis* against ferric sulfate were

significantly less than Chlorhexidine (p=0.05), But in the case of Lactobacillus acidophilus,

they had almost the same effect (p<0.05) (Table 2). .

Table 2. The antibacterial effect of the investigated substances on tested bacteria

Comparison of substances	bacteria	Statistic Z	p-*value*
Formocresols type 1 and 2	*streptococcus mutans*	1.12	0.261
	Enterococcus faecalis	1.964	0.050

	Lactobacillus acidophilus	1.77	0.077
Formocresol type 1 and Ferric sulfate	*streptococcus mutans*	1.964	0.050
	Enterococcus faecalis	1.964	0.050
	Lactobacillus acidophilus	1.964	0.050
Formocresol type 1 and Chlorhexidine	*streptococcus mutans*	1.964	0.050
	Enterococcus faecalis	1.964	0.050
	Lactobacillus acidophilus	1.964	0.050
Formocresol type 2 and Ferric sulfate	*streptococcus mutans*	1.964	0.050
	Enterococcus faecalis	1.964	0.050
	Lactobacillus acidophilus	1.964	0.050
Formocresol type 2 and Chlorhexidine	*streptococcus mutans*	1.964	0.050
	Enterococcus faecalis	1.964	0.050
	Lactobacillus acidophilus	1.964	0.050
Ferric sulfate and Chlorhexidine	*streptococcus mutans*	1.964	0.050
	Enterococcus faecalis	1.964	0.050
	Lactobacillus acidophilus	1.328	0.184

Discussion

Formocresol has been the gold standard for pulp treatment over the last 100 years and is widely recommended. However, in the last 20 years, its use has been doubted due to its systemic spread, inflammatory responses, and carcinogenicity (17). Thus, ferric sulfate was proposed as an alternative therapeutic option instead of formocresol (19). Despite the clinical success of formocresol and ferric sulfate, histological studies have shown severe inflammatory responses with formocresol and ferric sulfate pulpotomy (20). Thanks to its ease of use and antibacterial properties, formocresol has become the most popular pulp-covering substance (gold standard) for pulpotomy teeth with a success rate of 76-97% (21-23). However, there are concerns about the toxicity, mutagenicity, and potential carcinogenicity of this substance in humans (24, 25). For this reason, various substances such as ferric sulfate, calcium hydroxide, MTA, electrosurgery, and laser have been developed and tested for pulpotomy of primary teeth (17).

Ferric sulfate preserves as much living tissue as possible without inducing restorative dentin. Although its mechanism has not been fully clarified, it is believed that iron ions and ferric sulfate chemically react with blood proteins and cause agglutination of these proteins and the membrane of this complex mechanically covers the cut-off blood vessels and creates hemostasis and causes blood clot formation (18). Furthermore, previous studies have confirmed the antibacterial effect of ferric sulfate and formocresol (12, 13, and 26-30).

Based on the results of the present study, the highest mean no-growth zone diameter of the

investigated bacteria was related to formocresol type 2, followed by formocresol type 1. Also,

no significant difference was observed between the effects on the no-growth zone diameter in

the case of formocresol type 1 and 2. (except in the case of *Enterococcus faecalis*, which

showed a better result in formocrosol 2). In other words, the effect of formocresol type 1 and 2 on the no-growth zone diameter was the same, (except for *Enterococcus faecalis*) but a significant difference was observed between the effects of formocresol and ferric sulfate, indicating the greater effect of formocresol type 1 and 2 compared to ferric sulfate. In other words, formocresol was a more effective substance on the no-growth of the tested bacteria than other investigated substances.

In comparing the control group (0.2% chlorhexidine) with ferric sulfate, the results revealed a significant difference between the no-growth zone diameters of *S. mutans* and *E. faecalis*, but no significant difference was observed for *L. acidophilus*. In other words, the effect of chlorhexidine and ferric sulfate on inhibiting the growth of *L. acidophilus* is the same, but chlorhexidine has a much greater antimicrobial effect than ferric sulfate on *S. mutans* and *E. faecalis*.

In a study carried out by Meshki et al., results similar to the present study were obtained, while the formocresol had a much better effect on reducing the number of bacteria than the chlorhexidine group (27). In a study carried out by Youravong et al., silver nitrate had the most toxicity, while ferric sulfate had the lowest toxicity (30). Also, gram-positive species had a lower affinity for metals than gram-negative species (30). Also, Bandi et al. reported that ferric sulfate, as a local hemostatic agent and a common astringent solution (15.5%), was equally useful compared to other chemical hemostatic agents (26).

According to our search, few studies have compared the

antibacterial effects of formocresol and ferric sulfate. However, these substances are

extensively used in the treatment of pulpotomy but not as a specific

administration to control and killing of the bacteria (13). On the other hand, Chlorhexidine is

mostly used to clean and control oral bacteria (28). However, considering the

nature of pulpotomy treatment, which is the protection of deciduous teeth

with caries until permanent teeth grow in children

the results

obtained from previous studies that compared the success of pulpotomy with formocrosol and

ferric sulfate can be interpreted with the current results because the greater success of

formocrosol in the treatment of pulpotomy in previous studies may have been due to the

antibacterial properties of formocrosol.

In the studies conducted by Lele et al., Timpawat et al., and Tchaou et al., it was found that the antibacterial effect of formocresol is much greater than that of chlorhexidine (31-33), which is consistent with the results of the present study. However, in the study conducted by Al-Hyali et al., 0.2% Chlorhexidine was more effective on *E. faecalis* compared to the formocresol (34). The reason for this difference might be due to the difference in the methods of the studies.

On the other hand, Çinar et al. showed that ferric sulfate is a more effective antibacterial agent than Ankaferd Blood Stopper (ABS) on oral microorganisms (12). Moreover, Ismail et al. reported that ferric sulfate has a strong antibacterial effect on *S. aureus*, *Escherichia coli, Pseudomonas aeruginosa*, and *Serratia marcescens* but the preparation conditions significantly affect the antibacterial activity of ferric sulfate and the situation might be different in the oral environment (29). Also, Bandi et al. reported that ferric sulfate as a local hemostatic agent is equally useful compared to other hemostatic chemical agents and is extensively used in dentistry. However, its application in restorative dentistry and endodontics, pediatric dentistry, dental prosthesis, and for oral surgery has not been well documented (13). The results of the study conducted by Bandi et al. were inconsistent with the results of the present study since ferric sulfate showed the least effect on the inhibition of the bacteria.

Conclusion

According to the results of the present study and previous studies, it can be concluded that

formocresol has the greatest inhibitory effect on *S. mutans*, *E. faecalis*, and *L. acidophilus*.

Also, Formocresols type 1 and 2 had the same inhibitory effect, (except for *Enterococcus*

faecalis) but ferric sulfate was less effective. Furthermore, 0.2% Chlorhexidine showed

better antibacterial effects than ferric sulfate. (except for *Lactobacillus acidophilus*) Also, the

effect of all 3 substances on *S. mutans, E. faecalis*, and *L. acidophilus* was considerable,

indicating the good inhibitory effect of these agents. Considering the side effects of

formocresol including toxicity, mutagenicity, and carcinogenicity, the use of this agent in

clinical settings is problematic. Thus,

Considering these conditions, by using isolation, and in an aseptic

condition, the use of ferric sulfate can be recommended for pulpotomy of deciduous teeth.

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conflict of interests

The authors have avoided any plagiarism, misconduct, forgery or double posting and publication in compiling the research.

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All executive expenses during the study were carried out by the researchers and there is no financial right in any case.

Ethical statement

The principle of trustworthiness should be observed in the presentation of research sources.

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