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Research Article

Anti-Microbial Efficacy of Gingerols and Calcium Hydroxide Mixture Versus Metapex on Microorganism of Infected Root Canal of Primary Teeth

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Abstract

Aim: This study aimed to evaluate the antimicrobial efficacy of gingerols mixed with calcium hydroxide against microorganisms of infected pulp of primary teeth versus Metapex.

Methods: Microbial samples isolated from 20 necrotic primary root canals of 20 children were cultured in aerobic and anaerobic condition then by gram stain the microorganism colonies was distinguished to Gram+ve or Gram-ve. The inhibition zone of gingerols mixed with calcium hydroxide (Ginge-Cal) and Metapex was measured by the disc diffusion method.

Results: By using Mann Whitney test aerobic Gram +ve in the (G-a) group (12.86 ± 5.03 mm) while the mean value in the (M-a) group was (7.34 ± 4.59 mm) with a statistically significant difference between the two groups (P = 0.001). Aerobic Gram -ve in (G-a) group (10.69 ± 4.51 mm) while the mean value in (M-a) group was (6.48 ± 4.03 mm) significance difference between two groups (P = 0.039) with statistical significance difference. The result of an anaerobic Gram +ve and Gram -ve not show statistical significance difference.

Conclusion: The Ginge-Cal can be considered a promising material as pulpectomy paste or temporary inter-canal medicament for children's primary teeth with infected pulp.

Keywords: *Antimicrobial; Gingerols; Ginge-Cal; Infected Primary Teeth; Metapex*

Introduction

Infected teeth should be treated and preserved in a natural position in the dental arch, as long as they remain functional and disease-free again. As early as 1932, endodontics was advocated as a method of preserving baby teeth that would otherwise be lost [1]. Primary teeth with infected are multi microbial, with a predominance of bacteria, located deep in the dentin and cementum around the periapical area. These microorganisms can remain even after biomechanical preparation, complete debridement with antimicrobial rinse agents and intracanal dressings. Due to the task of complete debridement in terms of canal morphology and the immediate existence of permanent teeth, the obturation material must be biocompatible and eliminate these remaining germs [2]. It must neutralize its toxic products

and prevent reinfection of the canal to create a favorable environment for the healing process. Obturation materials that are used today in pulpectomy do not have all the required properties of an ideal pulpectomy filling material for primary teeth, especially concerning the major desirable property of having an anti-microbial effect (microorganism elimination) [3,4]. Also, the condition of the root canal, if it was exposed to oral fluids Microorganisms, food contamination, or it was reaching the canal as a result of the invagination of dental caries [5]. The presence of this large number of different bacterial species in the dental pulp canal of the infected tooth leads to necrosis of pulp tissue and accumulation of pus with a strong inflammatory reaction. For this reason, additional chemicals and mechanical approaches used as irrigation solutions, intracanal medicaments, rotary system, and lasers decrease the effect of the inflammation to preserve the primary teeth to perform their functions [6]. From previous studies that investigated the effectiveness of several pulpectomy materials and found that all obturation materials had an antibacterial effect but variation in grades [7,8]. In pulp therapy, there are several herbals such as Allium Sativum, Turmeric, Pomegranate, Green Tea, Neem, Cloves, and Aloe Vera [9]. That used as direct or indirect pulp dressing, pulpotomy filling, and pulp medicament. In the last few years, more scientific research has focused on the mechanisms and targets of ginger and its various components. Gingerols are the major powerful phenolic compounds that are used in some medical conditions as an antioxidant, anti-bacterial agent, anti-vomiting compound, anti-asthma, anti-nausea compound, and anti-cancer agent. In addition, it is inhabiting dementia, diabetes, cardiovascular disease, platelet aggregation, ulcerative colitis, and cholesterol [10,11]. Based on what was mentioned above, and the limited or lack of valuable studies to evaluate Gingerols in dentistry, especially in root canal treatment the present study focused on Gingerols and evaluate the possibility of using them in the elimination or decrease of microorganism in the infected necrotic pulp of primary teeth and compare it with Metapex.

Materials and Methods

This *in-vitro* study was carried out after obtaining the approval of the Ethics Committee of the Faculty of Dentistry Mansoura University with code number [06030718].

Preparation of a mixture of Gingerols - Calcium hydroxide (Ginge-Cal)

A semi-oily aqueous liquid of Gingerols was extracted in the laboratories of the Pharmacognosy Department, Faculty of Pharmacy, Mansoura University [12]. Gingerols, Calcium Hydroxide powder and Barium sulfate (radiopaque material) were mixed in approximately 3:2:1 ratio respectively and the mixture of the three materials was abbreviated Ginge-Cal. The collected samples were cultured and tested two materials in 2 main groups in different environmental conditions that were divided into 4 subgroups [13]. First group was Ginge-Cal group divided into (G-a): culturing in aerobic conditions and (G-ana): in anaerobic conditions; and the second group was Metapex group divided into (M-a): culturing in aerobic condition and (M-ana): in anaerobic condition.

Sample collection

After signing the consent from the child's guardian, the samples were isolated from 20 infected primary root canals of 20 children from the Clinical of Pediatric Dentistry Faculty of Dentistry, Mansoura University according to Gomes., *et al.* (2004) [14]. The children were 4 - 8 years old, did not use any antibiotics for at least two weeks, and had an infected primary tooth with soft tissue swelling or sinus tract. After rubber dam isolation and the surrounding field was decontaminated with a 3% Sodium hypochlorite solution for 30 seconds. The access cavity to the chamber and root canal orifice was done with sterile round burs without water spray. A # 20-25 sterile paper point was inserted in the distal canal of the lower primary molar to the approximate canal length for 60 seconds. A nutrient media in Eppendorf Safe-Lock tubes 2.0 ml was used to transport the samples to the laboratory in 5 - 10 minutes.

Sample culturing

Sample culturing was done in Microbiological Diagnostic and Infection Control Unit in the Faculty of Medicine, Mansoura University. Firstly, the sample from each child was immediately cultured on four coded Petri dishes, two of them containing Muller-Hinton agar and the other two containing Nutrient Blood agar. Each plate of the two different types of nutrient media was incubated under different environmental conditions, aerobic and anaerobic in an incubation oven for 48h at 37°C. The anaerobic environmental condition was carried out by placing cultured plates in an anaerobic -Jar with a Gas Pak system (Hydrogen gas (H_2) and Carbon Dioxide (CO_2) generating system) and sealed in an incubation oven at 37°C. The Gas Pak system work to replace the oxygen found in the atmosphere inside the jar and dissolved in the culture media. After 48 hours the growth of the microorganism (aerobic or anaerobic) is checked by the naked eye for each incubated petri dish (Figure 1). Microorganism colonies were distinguished by microorganism colony's morphology and Gram stain. The Gram stain test was accomplished under the microscope to confirm the type of microorganism depending on the color (Gram+ve blue/purple, Gram-ve pink/red). Depending on Gram stain examination the microorganism was cultured on new Muller-Hinton agar Petri dish for an antimicrobial susceptibility test.

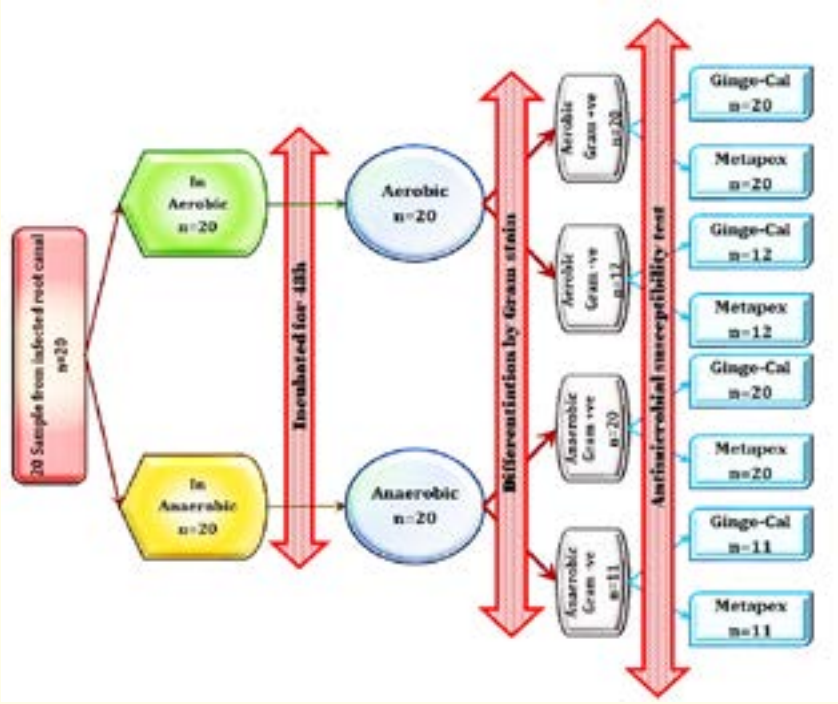

Figure 1: *A sequence diagram showing the distribution of samples cultured in aerobic and anaerobic conditions and the product after the process of distinguishing by gram stain.*

Antimicrobial susceptibility test activity

A 4 - 5 mm in diameter of Ginge-Cal and Metapex pasts was placed on a cultured Muller-Hinton agar petri dish and incubated for 24 hours in aerobic or anaerobic conditions. After 24 hours, the diameter of the inhibition zones around each material in the plates was measured by a digital caliper in millimeters. The average of three examiners' measurements was recorded. Then data were tabulated for statistically analyzed.

Results

Data were fed to the computer and analyzed using IBM SPSS software package version 27.0. (Armonk, NY: IBM Corp). Qualitative data were described using numbers. The significance of the obtained results was judged at the 5% level by the Mann-Whitney test. Twenty samples were isolated from 20 necrotic root canals of primary molar teeth of 20 children. Their age ranged from 4 - 8 years with a mean age of (6.08 ± 1.34). The samples were cultured on 40 Petri dishes under different environmental conditions, aerobic (n = 20) and anaerobic (n = 20). Every grew cultured sample distinguished by gram stain to Gram +ve (n = 20) with 100% and Gram -ve (n = 12) with 60% for aerobic culture, while Gram +ve (n = 20) with 100% and Gram -ve (n = 11) with 55% for anaerobic culture. The studied material Ginge-Cal and Metapex were evaluated by antimicrobial susceptibility test on 63 Petri dishes. The table 1 showed the Gram +ve in the (G-a) group (12.86 ± 5.03 mm) while the mean value in the (M-a) group was (7.34 ± 4.59mm) with a statistically significant difference between the two groups (P = 0.001). (Figure 2A). While table 2 showed the anaerobic Gram +ve and Gram -ve that did not show a statistical significance difference (Figure 2B). In comparison between the two studied materials that were cultured and grown in aerobic conditions, the high mean value of inhibition zone in the G-ana group Gram +ve was (12.97 ± 5.52 mm) while the low mean value of inhibition zone in the M-ana group was (8.87 ± 7.39 mm) with statistical significance difference between two groups (P = 0.108). In anaerobic Gram -ve the high mean value of the inhibition zone showed in the G-ana group was (8.14 ± 2.55 mm) while the inhibition zone means value in the M-ana group was (8.15 ± 4.62 mm) with no statistically significant difference between the two groups (P = 0.519). while when a comparison between the two studied materials that were cultured in aerobic and anaerobic conditions showed the low mean value of inhibition zone in Gram +ve G-a group was (12.86 ± 5.03 mm) while the mean value of inhibition zone in the G-ana group was (12.97 ± 5.52 mm) with no statistical significance difference between two groups (P = 0.841). The low mean value of the inhibition zone in the Gram +ve M-a group was (7.34 ± 4.59 mm) while the high mean value of the inhibition zone in the M-ana group was (8.87 ± 7.39 mm) with no significant difference between the two groups (P = 0.583).

Aerobic	Ginge-Cal. (G-a)	Metapex (M-a)	U	p
Gram +ve	**(n = 20)**	**(n = 20)**		
Min. - Max.	5.65 - 21.51	0.0 - 17.21	83.50*	0.001*
Mean ± SD.	12.86 ± 5.03	7.34 ± 4.59		
Median (IQR)	11.96 (8.86 - 17.04)	6.45 (3.62- 10.66)		
Gram -ve	**(n = 12)**	**(n = 12)**		
Min. - Max.	4.0 - 18.0	0.0 - 13.54	36.50*	0.039*
Mean ± SD.	10.69 ± 4.51	6.48 ± 4.03		
Median (IQR)	10.50 (7.18 - 14.11)	7.0 (3.98 - 9.03)		

Table 1: *Comparison between the two materials according to antimicrobial susceptibility test in Aerobic condition.*

*U: Mann-Whitney Test; IQR: Inter Quartile Range; SD: Standard Deviation; p: p-value for comparing the two studied groups; *: Statistically significant at p ≤ 0.05.*

Anaerobic	Ginge-Cal. (G-ana)	Metapex (M-ana)	U	p
Gram +ve	**(n = 20)**	**(n = 20)**		
Min. - Max.	0.0 - 22.0	0.0 - 19.07	140.0	0.108
Mean ± SD.	12.97 ± 5.52	8.87 ± 7.39		
Median (IQR)	12.99 (9.47 - 17.45)	9.58 (0.0 - 16.0)		
Gram -ve	**(n = 11)**	**(n = 11)**		
Min. - Max.	4.0 - 12.81	0.0 - 13.0	50.0	0.519
Mean ± SD.	8.14 ± 2.55	8.15 ± 4.62		
Median (IQR)	7.90 (6.55 - 9.77)	9.54 (6.30 - 11.66)		

Table 2: *Comparison between the two materials according to antimicrobial susceptibility test in Anaerobic condition.*

*U: Mann-Whitney Test; IQR: Inter Quartile Range; SD: Standard Deviation; p: p-value for comparing the two studied groups; *: Statistically significant at p ≤ 0.05.*

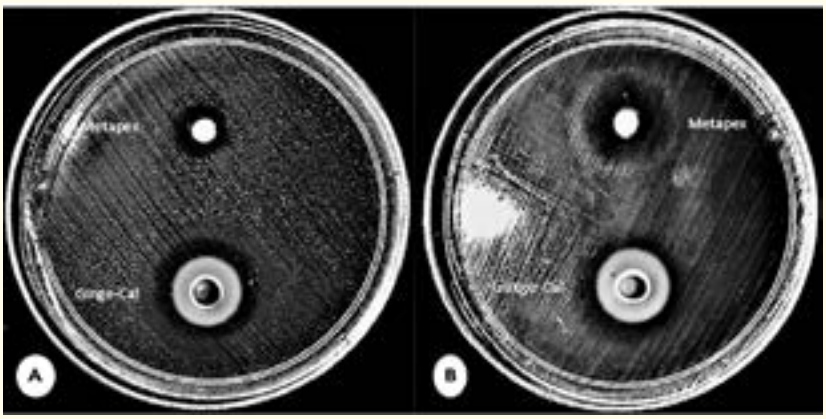

Figure 2: *Antimicrobial susceptibility test by disc diffusion method and the inhibition zone for studded material Ginge-Cal and Metapex (A) cultured in aerobic conditions, (B) cultured in anaerobic conditions.*

Discussion

Up to our knowledge, there are almost no articles in the literature that studied the effect of gingerols on the microbes in the infected pulp. This study was conducted to study the antimicrobial effect of gingerols as a natural material on the microorganism present in the infected primary pulp. the children were selected according to certain criteria. The most important of these criteria was the age and the child's non-use of antibiotics for two weeks before taking the sample. To prevent the large difference in the microorganism profile expected to be present in the pulp canal, and not to be affected by the type of antibiotics that are used and affecting on the microorganism growth. This is consistent with Kakoli., *et al*. [15] and Lee., *et al*. who confirmed that age is related to microbial variety in the inflamed pulp canal. This reasoning is also consistent with Shweta and Krishna [16] who confirmed that the response to antibiotics differs from one child to another due to the presence of some types of microbes that have formed immunity against antibiotics, and this leads to a difference in the effectiveness of the antibiotic against the microbial presentation in the infected root canal.

The present study observed a high main value of anti-microbial action of Ginge-Cal with a statistically significant difference when compared with Metapex on aerobic Gram +ve and aerobic Gram -ve. In addition, the anti-microbial action of Ginge-Cal on anaerobic Gram +ve showed high main value than Metapex without a statistically significant difference. While Metapex showed a high main value of anti-microbial effect on anaerobic Gram -ve than Ginge-Cal without a statistically significant difference. The result of Ginge-Cal on aerobic Gram +ve, aerobic Gram -ve, and anaerobic Gram +ve in this study is in agreement in terms of the effect of gingerols on microorganisms with Malu., *et al*. [17], Riaz., *et al*. [18] and Zhang., *et al*. [19] who mentioned the effectiveness of gingerols in its anti-microbial effect. Plus, calcium hydroxide was added, to enhance the synergistic action between calcium hydroxide and gingerols in antimicrobial action. Where several previous studies have proven that calcium has an antimicrobial effect [Agrawal] [20] and Ba-Hattab., *et al* [21]. Regarding the comparison of Gingerols with Metapex, the weak anti-bacterial effect of Metapex was observed by Harini., *et al*. [22] and Navit., *et al*. [23], Qasem., *et al*. [13] which is in agreement with the results of this study. This may be because iodoform has little or no antibacterial effect even though calcium hydroxide has been added [24]. As for the anaerobic Gram -ve result, this may be due to the decrease in the sample size produced after the screening process, which led to the convergence of the results between Metapex and gingerols, with a very slight increase in the results of Metapex with no statistically significant difference and this cannot be proven or denied because there are no previous studies in this regard.

Conclusion

Based on the present study results, we can conclude that Ginge-Cal can be considered a promising material as pulpectomy paste or temporary inter-canal medicament for primary teeth with infected pulp due to antimicrobial action. Clinical studies are needed to evaluate when used the material in pulpectomy paste or temporary inter-canal medicament for primary or permanent teeth with an infected root canal.

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Conflict of Interest

No any financial interest or any conflict of interest exists.

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