



Clinical and Antimicrobial Efficacy of Garlic Extract and Formcresol as a Non-vital Pulp Therapy Medicament in Primary Teeth

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KEYWORDS

*Pulpotomy, Antimicrobial,
Formacresol, Garlic extract.*

ABSTRACT

Aim: aim of the present study was to evaluate and compare the clinical and antimicrobial efficacy of garlic extract and formcresol as a non-vital pulp therapy medicament in primary teeth. **Subjects and methods:** The clinical study was conducted on 30 children of both sexes between the ages of 4 and 7 year. A total of 60 non-vital bilateral primary mandibular molars (first&second) were selected for nonvital (mortal) pulpotomy procedures at two visits: 7 days apart. Treated teeth are classified into two groups according to the drugs used in the treatment. The first group (control group) was dressed with formocresol, while the second group (study group) was dressed with garlic extract (GE). Microbiological samples were collected from each of the 60 specimens before treatment and 7 days later. **Results:** The results showed that pain disappeared in both groups significantly, with success rates of 95.2% and 95.6%, respectively. Other indicators were assessed with a 100% success rate of swelling and mobility for both groups. **Conclusion:** Clinical and microbiological analysis of this study revealed that there are potential natural alternative medicaments (GE) for formacresol, which can be considered equally effective.

INTRODUCTION

Preserving primary teeth is considered one of the necessities in the field of pediatric dentistry for many reasons; including the ability of the child to eat in a healthy way, preserve the arch length, psychology of the child, preventing the development of bad habits, as well as the natural growth of the jaws. The non-vital pulp treatment technique is one of the methods used to preserve primary teeth until the permanent teeth eruption.⁽¹⁾

Tooth decay is the main cause of tooth destruction and access to the pulp, which causes contamination with a wide variety of microbes. The microbial invasion causes irreversible changes of the pulp, formation of preapical abscesses and swelling of the gums, which can cause early loss of teeth. The primary root canal system facilitates the spread of

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microorganisms through lateral branches, numerous ramifications, and accessory root canals, which can cause damage to permanent successor. For these reasons, the complete removal of microbes from the root of primary teeth is exceedingly difficult.⁽²⁾

The pulp infections of primary teeth comprise of a complex mixture of different bacterial species. Anaerobic and aerobic as well as facultative anaerobic bacteria can be found in the primary root canal but the majority of anaerobes. Lactobacilli, Prevotella, Pepto streptococcus, Propionibacterium, Fusobacterium, and Eubacterium are the most anaerobic bacteria found.⁽³⁾ While; Streptococci, Staphylococci followed by gram negative bacilli as E.coli and Klebsiella are the commonly isolated aerobic bacteria (Itzhak et al, 2008)⁽⁴⁾. The treatment success depends on the ability to get rid of these massive quantities of microbes, which constitute the main obstacle in the treatment of the non-vital pulp therapy.

Non vital pulp therapy of primary teeth with necrotic pulp is routine in pediatric dentistry. Control of infection is mandatory because the close proximity of the developing permanent tooth germ to the roots of the primary teeth and because of the nature of bone favor dissemination of infection. So, it is fundamental that the Pediatric dentist be aware of the microbiota in these regions so that a strong and adequate antimicrobial agents may be used to eliminate these infections.

A major challenge in pediatric dentistry is selecting materials used in pulp therapy.⁽⁵⁾ Different materials have been used in the pulp therapy of deciduous teeth. Formocresol is widely used in pulp therapy of primary teeth with success rates 55 - 98%. due to its fixative and bacteriostatic properties (Zurn and Seale, 2008).⁽⁶⁾ The combined action of formaldehyde, an alkylating agent, and cresol, a protein coagulating phenolic compound, is highly antibacterial; but it may cause highly destruction of vital tissues.⁽⁷⁾

The immunological aspects, states that formocresol can function as a hapten, interact with the host protein, and result in an immunological reaction.⁽⁸⁾ As a result of the concern about these side effects of formocresol, a safe alternative was sought to avoid these effects (Antar et al, 2004).⁽⁹⁾

Herbal extracts and natural phytochemicals are promising to be used as alternatives to the chemical materials because of their high anti-microbial, anti-inflammatory, therapeutic effects, and biocompatible properties.⁽¹⁰⁾ Many hundreds of plants have been investigated for treatment of various bacterial infection. Allium sativum (Garlic extract) is one of the most extensively researched medicinal plants in the field of medicine and suggested to have anti-bacterial, anti-fungal, and anti-viral properties.⁽¹¹⁾ Garlic extract has a wide spectrum efficacy against gram positive and gram-negative microorganisms and also effective against resistant microorganisms to various drugs. Allicin is a compound released when cloves of garlic are crushed or chopped by the enzymatic action of alliinase (cysteine sulfoxide lyase) on alliin. This allicin compound and other sulfur derived components of garlic are believed to have therapeutic effects.⁽¹²⁾

The aim of the present study was to evaluate and compare the clinical and antimicrobial efficacy of garlic extract and formocresol as a non-vital pulp therapy medicament in primary teeth.

MATERIALS AND METHODS

Sativum extract preparation:

A 100 g of fresh natural garlic (*A. sativum*) was obtained, cleaned, peeled, and dried. It was then blended using sterilized mortar and pestle and squeezed using gauze. This extract was centrifuged at 10,000 rpm for 20 min and then filtered with a 0.45-um sterile disposable filter to obtain raw garlic extract (GE) and stored at -20°C until use.⁽¹³⁾



Clinical study:**Subject selection:**

The study was conducted in the Department of Pediatric Dentistry, Faculty of dentistry, Zagazig University. For this study, 30 children of both sexes were selected. The age of the patients ranged from 4-7 years and all of them are apparently healthy and cooperative according to Freckle behavior rating scale.

Inclusion criteria:

Clinical characteristics (Dentistry AAoP, 2009)⁽¹⁴⁾:

1. Non vital primary molars which may be asymptomatic or manifested with dull ache pain
2. Teeth with pathological mobility.
3. Restorable teeth
4. Percussion sensitivity
5. Swelling close to involved tooth accompanied with or without fistula

Radiographic characteristics of the root and supporting structures:^(15&16)

The extension of radiolucency at the furcation area did not exceed the half of the space between the furcation and the permanent successor. No internal root resorption or External root resorption.

Exclusion specifications

1. Uncooperative child and/or parents.
2. Unrestorable tooth
3. Presence of internal or external root resorption.
4. Presence of calcific metamorphosis inside root canals
5. Subjects who received antibiotic or anti-inflammatory drugs in the previous 2 months.

The treatment plan was explained in detail to the parents of the children and a written consent was

obtained before starting the study. Sixty bilateral first or second primary mandibular molars were selected.

Pulpotomy procedures:

Non vital pulpotomy (mortal pulpotomy) were done to the selected molars in two visits, 7 days apart.

First visit:

First, local anesthesia was administrated and isolation of the affected teeth with the rubber dam. The rubber dam and the teeth were cleaned using hydrogen peroxide solution (3%) and disinfected with sodium hypochlorite solution (2.5%). Preparation of the cavity and excavation of the carious tissues with large spoon excavator. Enlarge the cavity with round bur in low-speed hand piece, and then entire roof of the pulp chamber was removed. The coronal portion of the pulp tissues was removed using a large spoon excavator then the coronal pulp chamber was irrigated with saline 0.9% and dried.

Microbiological specimens were collected from each root canal of the 60 teeth by means of 2 sterile absorbent paper points under strict aseptic conditions. The paper points were introduced in the root canal, 1 mm shorter than the apical foramen and left for 1 minute to sucks the tissue fluid. From each tooth two specimens were collected; one to be cultivated aerobically was inserted in Stuart's transport medium (Conda lab, Spain), while the other to be cultivated an aerobically was inserted in anaerobic dental transport medium (Anaerobe systems, USA). All specimens were transported immediately to the laboratory of Microbiology and Medical Immunology Department, Faculty of Medicine, Zagazig University for microbiological evaluation.

After collection of all samples and according to the medicament used in treatment, the teeth classified randomly into two groups. Randomization was performed using the coin toss technique.

Group I: a small cotton pellet soaked in Buckley's formocresol* was placed in the pulp chamber over the root canals orifices.

Group II: a cotton pellet soaked in raw garlic extract (GE) was used then both cavities were filled by temporary filling materials* for 7 days.

Second visit:

After 7 days, all patients were clinically examined for presence of any signs or symptoms (1- pain, 2-swelling and 3- mobility) as a criterion for successful treatment. The temporary filling and the cotton pellet removed from the pulp chamber. The second specimens for the bacteriological investigation were taken from the treated canals of both groups as the first specimens and sent immediately to the microbiology lab. At the end antiseptic dressing (Formocresol or GE and eugenol mixed with zinc oxide powder) was placed and pressed down into root canals then the tooth was restored with a suitable restoration; Ibricevic & Al-Jame, 2003). In the second visit after one week, if any patient was presented with any symptoms, these teeth were retreated with the same medicament for another week until complete symptoms relief and then restored.

In vitro study:

Laboratory investigation:

Specimens in the anaerobic transport medium were inoculated on blood agar and immediately incubated in an anaerobic jar using anaerobic gas pack at 37°C for 72 hours. While specimens in the Stuart's transport medium were inoculated on blood, Chocolate and Mac-Conkey agar plates and incubated aerobically at 37°C for 24-48 hours. After 72 hours, bacteria grown an aerobically were sub cultured on blood agar and incubated aerobically for 24-48 hours. If no growth appeared on aerobic incubation, they were considered as obligate anaerobes. Gram stain, biochemical reactions, and colony morphology were used to identify the isolated bacteria, either aerobic or anaerobic.

Positive samples were reexamined after one week of treatment.⁽¹⁷⁾

Data were analyzed using the intention to treat analysis. Statistical analysis performed using the Qui-square test for binary variable. The level of significance was <0.05.

RESULTS

Out of 53 patients, 30 children with 60 bilateral deciduous molars with non-vital pulp were selected for the current study. Their age ranged from 4 to 7 years, with a mean (\pm SD) of 4.7 ± 7.1 years. The females represented 56.7%, while the males 43.3%. The mandibular second and first primary molars accounted for 55.4% and 44.6%, respectively. Thirty molars were treated with formocresol, while 30 molars on the other side were treated with garlic extract as a pulpotomy medicaments. All demographic data were demonstrated in Table 1.

Table (1) Distribution of demographic variables of participants

Gender	Group (1) n (%)	Group (2) n (%)	Total n (%)
Male	6 (20%)	7 (23.3%)	13 (43.3%)
Female	9 (30%)	8 (26.7%)	17 (56.7%)
Total	15 (50%)	15 (50%)	30 (100%)
Age (years) Mean \pm SD	4.6 \pm 6.7	4.8 \pm 7.5	4.7 \pm 7.1
First primary molars	14 (46.7%)	15 (50%)	29 (48.3%)
Second primary molars	16 (53.3%)	15 (50%)	31 (51.7%)
Total	30 (50%)	30 (50%)	60 (100%)

Clinical study

The evaluation after 1 week of treatment revealed that: all clinical symptoms were improved in both groups. The results showed that the pain disappeared in the two groups in a significant percentage, as there was only one case out of 21



remains in the first group, and one case out of 23 in the second group still felt pain, even if its intensity had decreased, with a success rate of 95.2% and 95.6%, respectively. As for the level of swelling and tooth mobility, they completely controlled in the two groups by 100% for both (Table 2).

Table (2) Distribution of pain, swelling and mobility in both groups.

Group	Time	Pain	Swelling	Mobility
Group 1 (FC) (n=30)	Preoperative	21	6	2
	Postoperative	1	-	-
Group 2 (GE) (n=30)	Preoperative	23	5	1
	Postoperative	1	-	-

Microbiological results:

In this study, there are more than ninety-six species of bacteria isolated from cultures that taken for samples before treatment from sixty molars with a rate 1.6 isolate/molar. The results of the isolated species showed that, most of them are aerobic and facultative anaerobic, representing a percentage of 67.7% (65 isolate), while obligatory anaerobic bacteria represent 32.3% (31 isolate). The isolated aerobic and facultative anaerobic bacterial species are composed of Pseudomonas 15 (23.1%), Staphylococci 15 (23.1%), Klebsiella 14 (21.5%), E.coli 13 (20%) and Streptococci 8 (12.3%) (table 3 and 4).

Table (3) Results of culture before treatment:

Culture findings	No. of samples
Total positive cultures	57
Total negative cultures	3
Positive for only aerobic microorganisms	15
Positive for only anaerobic microorganisms	9
Mixed for aerobic and anaerobic microorganisms	33
Total positive for aerobic microorganisms	48
Total positive for anaerobic microorganisms	42

Table (4) Types of isolated bacteria before treatment

Microorganisms	No. of specimens	No. of isolates
Aerobes only	15 (25%)	17 (17.7%)
Anaerobes only	9 (15%)	7 (7.3 %)
Mixed aerobes and anaerobes	33 (55%)	72 (75%)
No growth	3 (5%)	0
Total	60 (100%)	96 (100%)

After treatment, the results in table (5) showed that; the isolated bacteria were aerobic or facultative anaerobic, no obligate anaerobic bacteria were isolated. Table (6) showed that, in Garlic extract group, the isolated bacteria were completely controlled after one week of treatment in the number 43 culture (86%) of the infected molars. In the second group (Formocresol group), the recovery rate was 40 culture (83.3%) after the same treatment period.

Table (5) Types of isolated bacteria according to gram staining:

Culture findings	Aerobic	Anaerobic
Gram +ve cocci	19 (31.2%)	19 (54.3%)
Gram +ve bacilli	1 (1.6)	9 (25.7%)
Gram -ve cocci	0	0
Gram -ve bacilli	41 (67.2%)	7 (20.0%)
Total	61 (100%)	35 (100%)

Table (6) Culture results after treatment:

Treatment	Garlic extract	Formocresol
No. of teeth	30	30
No. of treated isolates	50	48
+ve cultures	7 (14.0%)	8 (16.7%)
-ve cultures	43 (86.0%)	40 (83.3%)
* Isolated bacteria	Staphylococci (49%) E. coli (32.3%) Klebsiella (18.7%)	Staphylococci (37%) E.coli (49.5%) Streptococci (13.5%)

DISCUSSION

The tendency to use plants began from ancient time and has been re-newed at recent years as alternatives to the synthetic medicines more than before because of their strong effect on overcoming many microbes; their affordability; their low side effects; their low toxicity; and the inability of many microbes to resist them.⁽¹⁸⁾

Non vital pulp therapy of primary molars aims to eliminate the microbes from the root canal system and to prevent the reinfections after recovery. The anatomical complexities of the root canal system like, lateral branches, numerous ramifications and accessory root canals makes the process of debridement of microbes mechanically extremely difficult. Therefore, the use of effective medicaments is necessary to complement the mechanical action thereby facilitating maximum removal of microorganisms.⁽¹⁹⁾

The treatment success of non-vital pulp in primary teeth depends on the ability to completely depletion or elimination of microorganisms, which enables the teeth to remain without clinical or radiological problems.⁽²⁰⁾

This study was conducted to analyze and compare the effectiveness of garlic extract and Formacresol medicaments against the viability of a complex mixture of bacterial species from clinical isolates.⁽²¹⁾

The results of clinical study showed that the Garlic extract was successful in more than 86% of the treated molars. This effect was very similar to the formocresol effect (83.3%). These results agree with the results of Riluwani et al. 2019.⁽²²⁾

These results may be due to the Allicin compound (the main active component of garlic extract), which is characterized by its ability to destroy the bacterial cell wall and cell membrane of root canal bacteria. Also, Allicin has ability to stop both spores' germination and Hovey's growth.

In the present study, aerobic, anaerobic and facultative anaerobic microorganisms, pseudomonas, streptococci, staphylococci, klebsiella and E.coli were found. These findings are in agreement with Toyoshima et al.⁽²³⁾ Who reported that, the microbiological investigation of necrotic pulp of primary teeth, revealed the presence of a polymicrobial infection with predominance of anaerobic microorganisms, similar to the microbiota of permanent teeth.

The results of this study showed that, 1.6 isolates were found per molar which enhance the microbial image of root canal infections, which corresponded to a study conducted by Silva et al, 2006.⁽²⁴⁾ This rate differs from the results of Brook et al, 1991⁽²⁵⁾, where 2.50 and 2.43 isolates per tooth were reported. Variation in rate may be due to the presence of negative cultures.

This study showed that, the most of isolated species are aerobic and facultative anaerobic which represents 67.7% of total isolates. These findings are consistent with the results of the studies conducted by Peciuliene et al, 2008⁽²⁶⁾ and Ambareen et al, 2015⁽²⁷⁾ were the methodology used in their studies was the same as that used in this study, also some of the root canals exposed directly to the oral environment. The remaining isolates represent 32.3% and consist of obligatory anaerobic bacteria. These results conflict with the results found by Sato et al.⁽²⁸⁾ Who reported more percentage of anaerobic than aerobic bacteria in primary teeth with necrotic pulp. The isolated aerobic and facultative anaerobic bacterial species are composed of Pseudomonas 15 (23.1%), Staphylococci 15 (23.1%), Klebsiella 14 (21.5%), E.coli 13 (20%) and Streptococci 8 (12.3%), which is consistent with the findings of Marsh and Largent.⁽²⁹⁾

According to the results of this study, gram positive cocci represented the main isolated anaerobic bacteria (54.3%). These results correspond to the many findings of previous studies that have been examined the nature of microbial colonization in root canal infections of primary teeth and reported



that the bacterial complex consisted of cocci and/ or rods, usually forming mixed species but cocci were the predominant one.

The present study showed that garlic extract when used as a non-vital medicament had a strong inhibitory effect against aerobic and anaerobic microorganisms (86%) isolated from necrotic dental pulp than formocresol (83.3%). This result is in accordance with Silvia et al, 2006 et al, 2010,⁽²⁵⁾ who found that garlic extract showed high antibacterial activity against all the tested bacteria species. The antimicrobial action of garlic extract is attributed to allicin whose mechanism of action partially inhibits DNA and protein synthesis, and entirely inhibits RNA synthesis. Concentrated garlic extract (95%) contains 34% allicin, 44% total thio sulfates, and 20% vinyl dithiols which is suggested to be responsible for antimicrobial activity.

CONCLUSION

The clinical and microbiological results of this study showed that, Garlic extract provides a good natural alternative to the synthetic medicaments to formocresol for treatment of non-vital primary teeth, because of their high antimicrobial, anti-inflammatory and biocompatible properties.

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الأزهر

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الفعالية السريرية ومضادات الميكروبات لمستخلص الثوم والفورمكريسول كدواء غير حيوي لعلاج اللب في الأسنان الأولية

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الملخص:

الهدف: ان الهدف من هذه الدراسة هو تقييم ومقارنة الفعالية السريرية ومضادات الميكروبات لمستخلص الثوم والفورمكريسول كدواء علاج اللب غير الحيوي في الأسنان الأولية.

المواد والأساليب: أجريت الدراسة السريرية على 30 طفلاً من كلا الجنسين تتراوح أعمارهم بين 4 و 7 سنوات. تم اختيار ما مجموعه 60 ضرساً فكيًا أوليًا ثنائيًا غير حيوي (الأولى والثانية) لإجراءات بضع اللب غير الحيوية (الميتة) في زيارتين: 7 أيام متباعدة. تصنف الأسنان المعالجة إلى مجموعتين حسب الأدوية المستخدمة في العلاج. المجموعة الأولى (المجموعة الضابطة) تم تزييفها بالفورموكرزول . بينما المجموعة الثانية (مجموعة الدراسة) تم تزييفها بمستخلص الثوم (GE). تم جمع العينات الميكروبيولوجية من كل عينة من 60 عينة قبل العلاج وبعد 7 أيام.

النتائج: وأظهرت النتائج أن الألم اختفى في كلا المجموعتين بشكل ملحوظ . حيث بلغت نسب النجاح 95.2% و 95.6% على التوالي. تم تقييم المؤشرات الأخرى بمعدل نجاح 100% من التورم والحركة لكلا المجموعتين.

الخلاصة: ظهر التحليل السريري والميكروبيولوجي لهذه الدراسة أن هناك أدوية بديلة طبيعية محتملة (GE) لالفورمكريسول . والتي يمكن اعتبارها فعالة بنفس القدر..

الكلمات المفتاحية: بتر عصب السن . مضادات الميكروبات . الفورمكريسول . مستخلص الثوم.