

Bacteriological evaluation of *Allium sativum* oil as a new medicament for pulpotomy of primary teeth

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Abstract

Objective: To compare the effects of *Allium sativum* oil and formocresol on the pulp tissue of the pulpotomized teeth. **Materials and Methods:** Twenty children were selected for this study. All children had a pair of non-vital primary molars. A sterile paper point was dipped in the root canals prior to the mortal pulpotomy. These paper points were collected in transfer media and immediately transported to the microbiological lab to be investigated microbiologically (for *Streptococcus mutans* and *Lactobacillus acidophilus*). Then the procedure of mortal pulpotomy was performed. After 2 weeks, the cotton pellets were removed and sterile paper points were dipped in the root canals for microbiological examination. Then comparison between the count of bacteria before and after treatment was conducted. Statistical analysis was performed using independent *t*-test and paired *t*-test at the significance level of $\alpha = 0.05$. **Results:** After application of both medicaments, there was a marked decrease in *S. mutans* and *L. acidophilus* counts. The difference between the mean of log values of the count before and after the application was highly significant for both medicaments ($P < 0.05$); however, better results were obtained when *A. sativum* oil was used. **Conclusion:** *A. sativum* oil had more powerful antimicrobial effects than formocresol on the bacteria of the infected root canals.

Key words: *Allium sativum*, formocresol, pulpotomy

INTRODUCTION

Preservation of the remaining vital portion of curiously exposed pulpal tissue in primary teeth, where the demand is to keep a functioning tooth in site, was one of the most frequent problems in pediatric dentistry.^[1] To solve this problem, pulpotomy therapy was introduced, developed, and classified according to treatment objectives.^[2] Pulpotomy involves amputation of the coronal portion of the affected or infected dental pulp. Treatment of the remaining vital radicular pulp

tissue surface should preserve the vitality and function of all or part of the remaining radicular portion of the pulp.^[3] Furthermore, it is an accepted procedure for treating both primary and permanent teeth with carious pulp exposures; several materials have been used for capping the radicular pulp after pulpotomy. These include formocresol (FC), glutaraldehyde,^[4] ferric sulfate,^[5] collagen material,^[6] and mineral trioxide aggregate.^[7] However, none of them had met the same degree of effectiveness and success rate as FC, because of the possible hazards of FC.^[8] Hazards such as cytotoxicity, carcinogenicity, and immunologic, biochemical, mutagenic, and teratogenic changes have been reported in the host.^[9] Moreover, it produced enamel defects in the permanent successors.^[10] A systemic uptake of FC has been found in pulpotomized teeth.^[11] The tissue changes have been found to occur in various internal organs, particularly in kidney and liver. The quantity of circulating FC was found to increase with the number of teeth treated.^[12] Hence,

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increased utilization of indigenous plant medicines in developing countries became a policy of the World Health Organization in the 1970s.^[13]

In this regard, a demand for natural medicament to replace FC as a pulp dressing material became imperative. Historically, garlic has been used around the world to treat many conditions, including hypertension and infections. Currently, garlic is used for reducing the cholesterol levels and cardiovascular risk, as well as for its antineoplastic and antimicrobial properties.^[14]

Allium sativum is one of the most extensively researched medicinal plants and its antibacterial activity depends on allicin produced by the enzymatic activity of alliinase (a cysteine sulfoxide lyase). Allicin and other thiosulfonates are believed to be responsible for the range of therapeutic effects reported for garlic; there is extensive literature on the antibacterial effects of fresh garlic extract.^[15] Garlic extract has been reported to inhibit the growth of various Gram-positive and Gram-negative bacteria.^[16]

A. sativum extract has been known to have inhibitory activity on various pathogenic bacteria, viruses, and fungi. Inhibitory activity of garlic extract has been demonstrated on multidrug-resistant (MDR) strains of *Streptococcus mutans* isolated from human carious teeth.^[17] Interest in medicinal plants has burgeoned due to increased efficacy of new plant-derived drugs and the growing interest in natural products. Because of the concerns about the side effects of conventional medicine, the use of natural products as an alternative to conventional treatment in the healing and treatment of various diseases has been on the rise in the last few decades.^[18]

Aim of the study

The aim of this study was to compare the microbiological effects of *A. sativum* oil and those of FC in mortal pulpotomy of primary teeth.

MATERIALS AND METHODS

A total of 20 children ranging in age from 4 to 8 years were selected from the outpatient clinic of Pediatric Dentistry Department, Faculty of Dental Medicine, Al-Azhar University, Egypt. Each one of these children had a pair of non-vital primary molars (upper or lower second primary molars).

Ethics of the study

- Care giver approval was taken
- Approval of Faculty of Oral and Dental Medicine, Al-Azhar University (under number 249/2010) was obtained.

Inclusion criteria

- Patient and parent cooperation
- Absence of any systemic disease which would contraindicate pulp therapy
- No previous history of antibiotic therapy for at least 2 weeks
- Presence of clinical signs or symptoms suggesting a non-vital tooth, such as localized swelling, suppurating sinus, tenderness on percussion, or slight mobility
- Possibility of establishing a final restoration of the tooth.

A rubber dam was used to isolate the designated molar. Cavity outline was established with a sterile # 330 high-speed, pear-shaped carbide bur with air/water spray. Caries was removed with slow-speed, sterile, round steel burs. Access to pulp chamber could be detected with a probe. The roof of the pulp chamber was removed with a sterile, non-end cutting, slow-speed bur. Removal of the coronal pulp tissue was achieved with a sterile, low-speed carbide round bur No. 6 and/or sharp, large, spoon excavator. A sterile paper point was dipped in the root canals prior to the mortal pulpotomy technique. These paper points were collected in transfer media that were immediately transported to the microbiological lab in order to be investigated microbiologically (*S. mutans*, *Lactobacillus acidophilus*).

Microbiological evaluation

Microbial samples were taken before and after application of *A. sativum* oil and FC for evaluation and comparison of the antimicrobial efficacy of the studied medicaments. Samples were obtained with sterile absorbent paper points and transferred into screw-capped tubes containing 5 ml of transport medium "Amic's transport medium."

The medium was then sterilized by autoclaving at 121°C for 15 min. The specimens were immediately transported to the microbiological lab on a transport medium. The transport medium containing the

specimens was dispersed by agitation in a vortex mixer at maximum speed for 60 s. One milliliter aliquots were aseptically spread with a sterile bent glass rod on the proper medium for each organism.

Media for culturing of clinical specimens:

- Mitis salivarius agar is the medium selected for isolation of *S. mutans*
- Tomato agar medium used for isolation of lactobacilli.

The ingredients were heated to dissolve the components, autoclaved at 121°C for 15 min, and left to cool. Approximately 20 ml of the medium was poured into each petri plate and left to solidify at room temperature, then stored in the refrigerator at 4°C until used. The inoculated plates were then placed in anaerobic jar containing gas pack and incubated for 2–4 days at 37°C.

Bacterial count

Bacterial colonies were counted using light microscope and expressed as colony forming units/ml (cfu/ml) [Figures 1 and 2].

Then, the procedure of mortal pulpotomy was continued by placing cotton pellets dipped in *A. sativum* oil [Captin Company (CAPpharm) registration No 952/94 Cairo, Egypt] and in FC (Petrópolis-RJ-Industria Brasileira, Dentsply, Latin America) over the root canals' orifices. After that, the tooth was filled with temporary filling containing the hard mix of zinc oxide and eugenol. After 2 weeks, the temporary filling and the cotton pellets were removed and then sterile paper points were dipped in the root

canals for microbiological examination. Comparison between the count of bacteria before and after treatment was made.

Statistical analysis

Statistical analysis was performed using independent *t*-test and paired *t*-test at the significance level of $\alpha = 0.05$.

RESULTS

Identification studies on the bacterial isolates

The results of this study indicated that the microbe of the infected root canals is mixed, comprising a variety of microorganisms. However, the most frequently isolated microbes were *S. mutans* and *L. acidophilus*.

Identification criteria of the isolate belonging to *S. mutans*

Colonies of the isolated bacteria appeared hyaline, slimy, and non-pigmented on mitis salivarius agar medium plates [Figure 1]. They were Gram-positive cocci, non-spore formers, and without resting stage. The cells appeared in pairs and chains. Further analysis indicated that the metabolism of the isolated bacteria was respiratory and fermentative. The isolate was able to grow in the presence of 40% bile salt or 4% NaCl. It was able to ferment large number of carbohydrates such as trehalose, D-mannose, D-glucose, D-fructose, lactose, maltose, sucrose, melibiose, raffinose, starch, inulin, sorbitol, mannitol, and *m*-inositol and produce acids. The previous findings indicated that the isolate was ranked to be *S. mutans*.

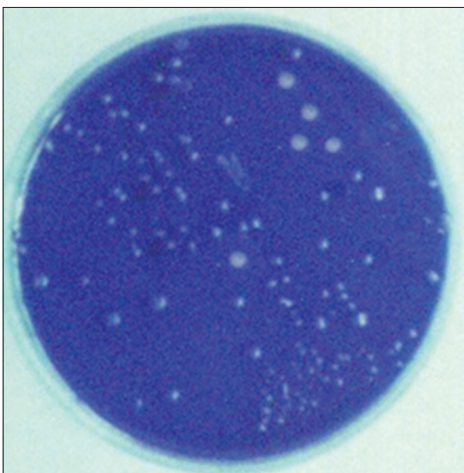


Figure 1: A photograph showing the growth of *Streptococcus mutans* on mitis salivarius agar medium

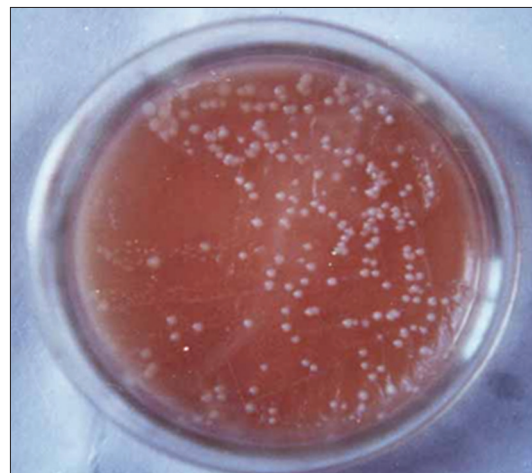


Figure 2: A photograph showing the growth of *Lactobacillus acidophilus* on tomato agar medium

Identification criteria of the isolate which belonged to *L. acidophilus*

Colonies of the isolated bacteria appeared creamy, glistening, and opaque without pigments on tomato agar medium [Figure 2]. The colonies were not slimy. They did not develop characteristic odor. The isolate had Gram-positive reaction, rod-shaped appearance, and were non-spore formers. Metabolism was fermentative only. The colonies did not produce catalase or cytochrome oxidase. The isolate was not able to produce H₂S. Its growth was not recorded at 15°C and arginine dihydrolase was negative. It was able to ferment large number of carbohydrates such as glucose, galactose, fructose, lactose, maltose, sucrose, and starch and produce acids. Meanwhile, it did not ferment pentoses such as xylose, arabinose, ribose, rhamnose, and trehalose, in addition to melibiose, melezitose, mannitol, sorbitol, and raffinose. The previous findings indicated that the isolate had *L. acidophilus*.

Bacterial count

After application of both medicaments, there was a marked decrease in *S. mutans* count [Figure 3]. The difference between the mean of log values of the count before and after the application was highly significant for both medicaments ($P < 0.05$). However, the antibacterial effect of *A. sativum* oil was more than that of FC [Table 1].

A noticeable decrease in *L. acidophilus* count was found after application of both medicaments [Table 2] and [Figure 4]. The difference between the mean of log values of the count before and after the application was highly significant. However, the antibacterial effect of *A. sativum* oil was more than that of FC.

DISCUSSION

Treatment of the remaining radicular pulp tissue involves amputation of the coronal portion of the affected or infected dental pulp. Furthermore, it is an accepted procedure for treating both primary and permanent teeth with carious pulp exposures. Pulpotomy has become the dominating pulp therapy for deciduous dentition because of the complicated anatomy of the root canals in primary teeth, the proximity of the permanent tooth germ, and the difficulties in finding a root canal filling material compatible with physiological root resorption.^[19]

Table 1: Comparison of *Streptococcus mutans* count before and after treatment with *allium sativum* oil and formocresol

	N=10			
	<i>Allium Sativum</i> Oil		Formocresol	
	Mean	SD	Mean	SD
<i>Streptococcus mutans</i> count				
Before	4843.00	23.05	4774.00	44.10
After	1505.48	6.12	1661.89	8.89

Table 2: Comparison of *Lactobacillus acidophilus* count before and after treatment with *allium sativum* oil and formocresol

	N=10			
	<i>Allium sativum</i> oil		Formocresol	
	Mean	SD	Mean	SD
<i>Lactobacillus acidophilus</i>				
Before	3536.00	17.08	3641.00	21.50
After	1302.49	9.14	1366.70	5.34

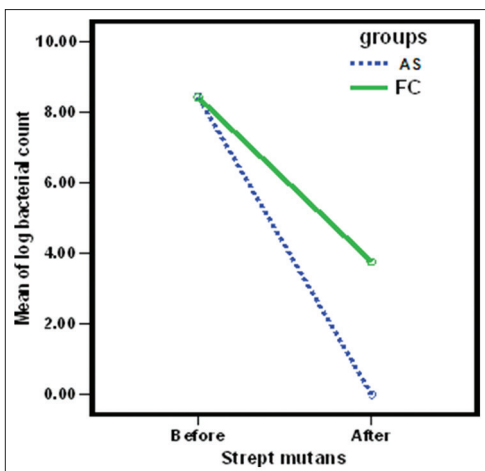


Figure 3: Comparison of *Streptococcus mutans* count before and after treatment with *Allium sativum* oil (AS) and formocresol (FC)

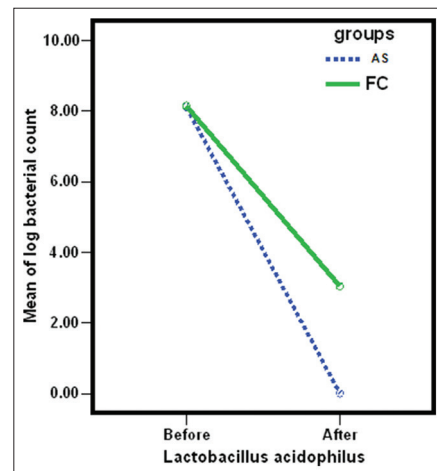


Figure 4: Comparison of *Lactobacillus acidophilus* count before and after treatment with *Allium sativum* oil (AS) and formocresol (FC)

A number of studies have been conducted on this alternative therapeutic system as well as its use for several purposes. It serves as a therapeutic alternative, safer choice, or in some cases, as the only effective treatment. People belonging to different cultures and places are known to have used the same plants for similar medical problems.^[18]

Nigella sativa oil (NS) extracted from black seed was used in pulpotomies for dogs in a study. In this study, a histopathological comparison of FC and NS pulpotomies was conducted in dogs. Specimens in NS groups showed mild to moderate vasodilatation and continuous odontoblastic layer, and few samples showed scattered inflammatory cell infiltration.^[20]

In spite of the large number of pharmacological studies carried out worldwide on *A. sativum*,^[21] the scrutiny of the published articles showed that there is a need to investigate its application in dental practice. This study, therefore, was the first to find out the effect of *A. sativum* in the treatment of pulpal affected primary teeth.

Teeth included in the present study were non-vital primary molars in which the infected coronal pulp remained, and hence, microorganisms invaded the radicular pulp resulting in irreversible pulpitis and necrosis. At this stage, the radicular pulp is unable to recover and the preferred treatment is pulpectomy.^[22] However, pulpectomy of primary molars is often impractical because of the difficulty in having adequate access to the root canals in small mouth openings of children, as well as the complexity of the root canals (ribbon shaped, lateral branching, and ramifications). Therefore, non-vital (mortal) pulpotomy technique is often used and preferred by clinicians.^[23] The microbiological investigations of the present study showed that there were microorganisms in the infected root canals.^[24] This result is concomitant with several studies.^[25] Microbiological results also revealed that *A. sativum* oil and FC possess antimicrobial activity as evidenced by marked decrease in the bacterial count after their application. So, the mechanism by which *A. sativum* can have an effect on pulp is through its antibacterial activity.

In the present study, the antibacterial effect of FC was strong against *S. mutans* and *L. acidophilus*. This was evident by the decrease in their count after administration of FC.^[26] This result is in concomitant with another study.^[27] FC has strong antimicrobial activity.^[28]

Comparison of drop percentage in different bacterial species counts revealed variability in the response of the microorganisms to FC. The most sensitive microorganism was *L. acidophilus*. On the other hand, the most sensitive microorganisms to *A. sativum* oil were *S. mutans* and *L. acidophilus*. The antibacterial effect of *A. sativum* oil on the previously mentioned microbes was greater than that of FC. This finding is in agreement with a previous study.^[29]

The antibacterial effect of *A. sativum* oil is due to the presence of a variety of compounds including ajoene, a garlic-derived sulfur-containing compound and Allicin exerted.^[21] The anti-inflammatory effect of *A. sativum* has been reported by several investigators.^[30]

It inhibits prostaglandins through the suppression of cyclooxygenase (COX) enzyme in the inflamed area and inflammatory cytokines. In a fashion similar to the action of COX-2-specific non-steroidal anti-inflammatory drugs, it also inhibits prostaglandins through suppression of 5-lipoxygenase enzyme. In addition, allicin and ajoene appear to inhibit inducible nitric oxide synthase in macrophages.^[31]

CONCLUSION

- *A. sativum* oil has potent antibacterial properties that enable it to combat intracanal microbes in the infected pulp of primary molars
- As the usage of *A. sativum* oil in pulp treatment of vital and non-vital primary molars was promising, it would encourage the dentists to use it in the future as an alternative to FC.

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