

Allium Sativum Oil as an Alternative Non-Vital Pulpotomy Medicament in Primary Teeth - A Randomised Controlled Trial

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ABSTRACT

Objective: To use *Allium sativum* oil as non-vital pulpotomy medicament in primary teeth by evaluating its antibacterial effect (Colony-Forming Units/ml- CFU/ml), against *Streptococcus mutans* and *Lactobacillus acidophilus*.

Study Design: A double-blinded, randomised controlled trial.

Place and Duration of the Study: Paediatric Dentistry Department, de' Montmorency College of Dentistry, Lahore in collaboration with the Microbiology Department, Lahore General Hospital, from October 2022 to February 2023.

Methodology: Forty patients aged between 4 to 8 years, each containing at least one non-vital primary molar, were randomly divided into Group A (Formocresol) and Group B (*Allium sativum* oil) using the lottery method. Non-vital pulpotomy (NVP) was performed by removing the coronal necrotic pulp. Sterile paper points were dipped in the root canals and taken to the laboratory. Cotton pellets soaked in the respective medicaments were placed over the root canal orifices and filled temporarily. Patients were recalled after one week. Samples were again taken, and the tooth was restored. Comparison was made between bacterial count at baseline and after one week of treatment, and it was expressed as CFU/ml.

Results: There was a significant reduction in median *Streptococcus mutans* and *Lactobacillus acidophilus* bacterial count in each group after one week of treatment ($p < 0.001$). Formocresol showed a higher average reduction (30300 ± 14060) compared to *Allium sativum* oil (24850 ± 9121). However, statistically, the difference was insignificant ($p = 0.314$) indicating both the medicaments possessed comparable antibacterial effects.

Conclusion: *Allium sativum* oil was found an effective alternative to Formocresol.

Key Words: Formocresol, *Allium sativum*, Non-vital pulpotomy, Primary teeth, Randomised controlled trial.

How to cite this article: Mazhar M, Naz S, Zahra A, Bashir N, Hussain MA. *Allium Sativum* Oil as an Alternative Non-Vital Pulpotomy Medicament in Primary Teeth - A Randomised Controlled Trial. *J Coll Physicians Surg Pak* 2024; **34(03)**:267-271.

INTRODUCTION

Preservation of cariously exposed primary teeth till their physiological exfoliation is one of the major problems in paediatric dentistry.¹ If primary teeth are left untreated, bacterial invasion from the coronal to the radicular pulp and then beyond the root canal into the adjacent soft tissue occurs. This leads to aggressive and painful inflammatory reaction rendering the tooth irreversibly inflamed or non-vital.² Retention of primary teeth is imperative as their untimely loss can lead to undesirable consequences.³

Bacteria present in primary teeth with necrosed pulp are polymicrobial, predominantly *Streptococcus mutans*, and *Lactobacillus acidophilus*.⁴ *S. mutans* is commonly regarded as the dental caries initiator,⁵ and *L. acidophilus* as dental caries progressor.⁶

Treatment of choice to save cariously exposed non-vital yet restorable primary teeth is pulpectomy, as the damaged pulp is unable to heal itself.⁷ Despite its high success rate, pulpectomy is often impractical in some paediatric patients, such as, when there are difficulties in obtaining adequate access due to small mouth openings in children, severe gag-reflex, possible damage to permanent tooth germ, complex (ribbon-shaped) non-negotiable root canals, and limited patient cooperation.^{1,7} Extraction is another option for the management of non-vital primary tooth, where the tooth is non-restorable due to extensive caries and with severe mobility.⁸

Non-vital pulpotomy (NVP) also known as Mortal pulpotomy, is regarded as a quick and least invasive alternative with predict-

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Received: July 30, 2023; Revised: January 21, 2024;

Accepted: February 28, 2024

DOI: <https://doi.org/10.29271/jcpsp.2024.03.267>

able outcomes for the decayed but restorable primary teeth that are non-vital. The procedure is performed when neither pulpectomy nor extraction is feasible. In NVP, the infected non-vital radicular pulp is dressed with a strong antiseptic material to combat infection, mummify, fix, and sterile the necrotic pulp tissue, so that it can stay *in situ* till the tooth exfoliates.⁹

Several conventional materials have been used in pulpotomy, commonly Mineral Trioxide Aggregate (MTA),¹⁰ Glutaraldehyde, and Ferric sulfate. However, the 'gold standard' material due to its potent antibacterial and fixative properties is Formocresol. Its constituents, formaldehyde and cresol, are responsible for harmful effects¹¹ such as mutagenicity, carcinogenicity, allergenicity, systemic distribution, tissue changes in internal organs, and enamel defects in permanent successors.¹

Several herbal medicaments have been used in pulpotomy procedures in paediatric patients because of their wide therapeutic properties, minimum side effects, low cost, easy availability, and long-lasting therapeutic effects with the added benefit of minimum bacterial resistance.¹² Garlic (*Allium sativum*) possesses potent antibacterial potential,¹² being anti-inflammatory, analgesic, antioxidant, and anti-cariogenic.¹³ It is effective against both gram-positive and gram-negative bacteria and has 1000-fold less bacterial resistance than other antibiotics.¹² There is literature on *A. sativum* use as vital pulpotomy medicament,¹⁴ but very limited literature as a non-vital pulpotomy medicament.^{1,7}

There is a lack of international literature, and no local study could be found on non-vital pulpotomy procedure and the medicaments used in it. The purpose of the study was to find a suitable herbal alternative (such as *Allium sativum* oil) to replace the routinely used conventional medicament, Formocresol, in the non-vital pulpotomy procedures.

METHODOLOGY

It was a double blinded, randomised controlled trial, conducted at the Paediatric Dentistry Department, de' Montmorency College of Dentistry/Punjab Dental Hospital, Lahore in collaboration with the Microbiology Department, Lahore General Hospital, from October 2022 to February 2023. Ethical approval was obtained from the Ethical Review Committee, Post-Graduate Medical Institute/ Ameer-ud-Din Medical College/ Lahore General Hospital, Lahore (AMC/ PGMI/ LGH/ Synopsis No./ 0081-19). The sample size was calculated using WHO Calculator version 12.2.6.

Forty cooperative patients of either gender aged between 4 to 8 years, were selected after the consent of parents, with ASA I and II, no history of antibiotic use for 2 weeks, and having forty non-traumatic, cariously exposed non-vital primary molars (one tooth from each patient), from the Outpatient Department of Paedodontics, de' Montmorency College of Dentistry, Lahore. Patients with history of allergy to anaesthetics and latex, radiographic evidence of pulpal floor opening into the furcation area, and with more than half of the roots resorbed were excluded.

Participants were enrolled according to their eligibility criteria and randomly allocated into Group A (Formocresol, control group-standard medicament, n=20) and Group B (*Allium sativum* oil, n=20) using the lottery method. The participants were blinded against which medicament group they belonged. Premade Formocresol (Tricresol and Formalin) was used composed of Tricresol 35%, Formaldehyde (40%) 19%, and excipient additive 100%. Likewise, premade *Allium sativum* oil (Garlic oil)⁷ was used.

For ease of procedure, local anaesthesia with 2% lidocaine containing 1:100000 epinephrine was injected, and a rubber dam was applied. Cavity outline and caries removal were done using #330 pear-shaped carbide bur using a high-speed hand-piece with constant water spray. Pulp chamber was accessed and de-roofing was done using non-end cutting steel straight fissure bur. Removal of the coronal pulp tissue (if any) was achieved with a sterile, sharp spoon excavator Type-A.⁷

The samples were collected for *in-vitro* evaluation. One sample was taken from each root canal by dipping #20 sterile paper point 2-3mm into the root canal for 10 seconds. These paper points were then immediately placed into plastic calibrated screw-capped tubes containing 5ml of autoclaved Fluid Thioglycollate Transport Medium,¹⁵ labelled with a laboratory code and transported to the lab as soon as possible.⁷

The radicular pulp was dressed with #4 (3mm) cotton pellet which was dipped in respective medicament. The tooth was then restored temporarily with a thin layer of Cavit followed by a thick hard mix of Zinc Oxide and Eugenol (ZOE) type II. The patient was then recalled after one week. Again, sample collection was done and transported to the lab. Additionally, the patient was clinically assessed according to standard criteria.⁷

Blood agar was used for culturing *S. mutans*, while de Man, Rogosa, Sharpe (MRS) agar was used for *L. acidophilus*. Culture media plates were prepared according to the manufacturer's instructions by a laboratory technician. Tubes containing the specimen were vortexed for 60 seconds. One µl portion of the sample was taken with one µl disposable plastic loop and spread on respective culture media plate for quantitative analysis.¹⁶ The inoculated plates were then placed in an anaerobic jar with a gas pack and incubated for 2 days (48 hours) at 37°C.⁷

Bacterial colonies were identified by the naked eye, gram-staining and catalase test. Further identification was done under light microscope with 100X magnification. For species level identification, Analytical Profile Index kit, API 20 STREP kit, and API 50 CHL kit were used for *S. mutans* and *L. acidophilus* identification, respectively according to the company's instructions. The results were entered in licensed API software with database V7.0 for *S. mutans* and V5.1 for *L. acidophilus* for interpretation.

To quantify antibacterial effect, *S. mutans* and *L. acidophilus* colonies counted with the naked eye were expressed as CFU/ml.

The study was set at 90% power and 95% confidence level with a 5% margin of error. The normality of data was assessed by the Shapiro-Wilk test. Mann-Whitney U test was used for the comparison of bacterial count among different groups. Fisher's exact test was used to compare clinical outcomes among different groups. $p \leq 0.05$ was considered as significant.

RESULTS

Bacterial colonies of the *S. mutans* appeared greyish-white, smooth, glossy, and translucent with zones of alpha hemolysis (partial hemolysis with greenish-brown discoloration) on blood agar plates (Figure 1 a,b). They were gram-positive, catalase-negative, and appeared as cocci-shaped, present in pairs and chains, without spores under a light microscope (Figure 1c). Bacterial colonies of *L. acidophilus* appeared compact, smooth, medium, opaque, and white mucoid on the MRS agar plate (Figure 2 a,b). They were gram-positive, catalase-negative, and appeared as round-ended single, pairs, and chains of rods without spores under a light microscope (Figure 2c). Further biochemical testing using API 20 STREP kit for *S. mutans* and API 50 CHL kit for *L. acidophilus* was done, and the results were analysed through API software.

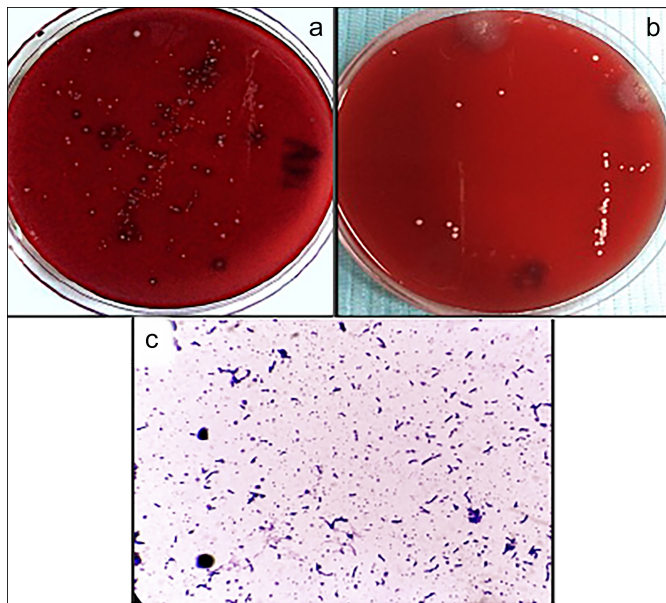


Figure 1: *S. mutans* colonies on blood agar plate, (a) Polymicrobial cultures at baseline with suspected *S. mutans* colonies (yellow arrow), (b) Bacterial cultures after one week of medicament placement, (c) Microscopic view of *S. mutans*.

The data were not normally distributed according to Shapiro-Wilk test, so median bacterial count for both the groups was calculated. There was statistically no significant difference in the average reduction of *S. mutans* as well as *L. acidophilus* bacterial count among the groups according to Mann-Whitney U test. For analysis of combined bacterial count among the groups, the mean bacterial count for both the groups was calculated, as the data were normally distributed. Results of Mann-Whitney U test showed statistically no significant difference in the mean reduction of combined bacterial count among the groups ($p = 0.314$, Table I) indicating that the overall antibacte-

rial effect of both the medicaments was similar. Clinical outcome was analysed through Fisher's exact test which revealed that there was statistically insignificant difference in the clinical outcome among the two groups ($p > 0.999$, Table II).

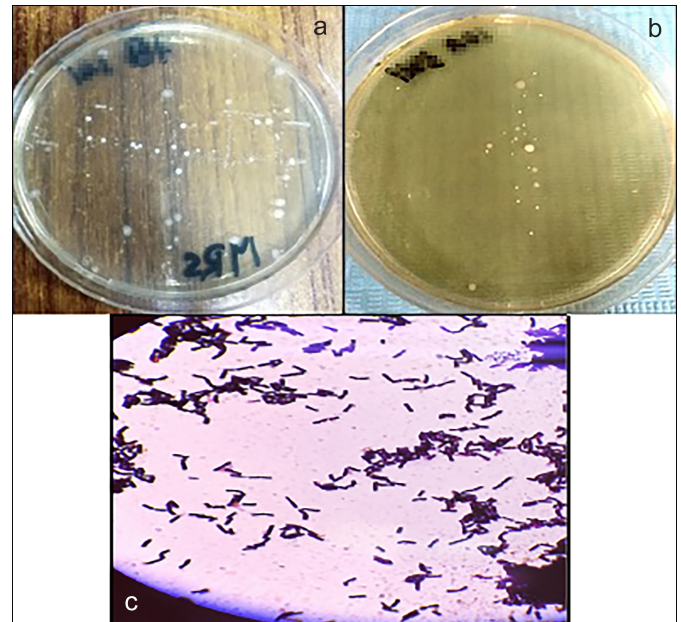


Figure 2: *L. acidophilus* on MRS agar plate, (a) Polymicrobial cultures at baseline with suspected *L. acidophilus* colonies (yellow arrow), (b) Bacterial cultures after one week of medicament placement, (c) Microscopic view of *L. acidophilus*.

Table I: Comparison of mean reduction of combined bacterial count among groups.

Group	Mean reduction of overall bacterial count (CFU/ml)			
	Mean ± SD	Minimum	Maximum	p-value #
Group A	30300 ± 14060	13000	60000	0.314
Group B	24850 ± 9121	10000	43000	

Group A: Formocresol, Group B: Allium sativum oil, # Mann-Whitney U test.

Table II: Comparison between the effects of all the three medicaments on the clinical outcome of non-vital primary teeth after one week (1st follow-up visit).

Clinical findings after one week	Group A [n = 20 (%)]	Group B [n = 20 (%)]	p-value*
Postoperative pain	(+) 2 (10.0%)	-	>0.999
Tenderness to percussion	-	(+) 1 (5.0%)	
Gingival inflammation	-	-	
Draining sinus	-	-	
Increased mobility (Grade II and III)	-	-	

Group A: Formocresol, Group B: Allium sativum oil, * Fisher's exact test. + (having signs and symptoms), - (without signs and symptoms).

DISCUSSION

The study was designed to evaluate the antibacterial effect of Formocresol (Group A), and Allium sativum oil (Group B) against *S. mutans* and *L. acidophilus*. These bacteria are the main cause of tooth decay, leading to irreversible damage and, hence, necrosis of tooth. A combined clinical laboratory study was performed to assess the clinical outcome as well of the antibacterial effects of each medicament.

Non-vital (Mortal) pulpotomy procedure was opted in this study as it is often used as a quick, least invasive alternative for

decayed, irreversibly inflamed/ non-vital, yet restorable primary teeth. It is performed when neither pulpectomy nor extraction is feasible.⁹ Pulpectomy is impractical in some paediatric patients with inadequate access, limited cooperation, and possible damage to permanent tooth germ.^{1,7} Extraction is another option, but it is an invasive procedure and can lead to space maintenance issues.¹⁷

The most significant finding of the present study was that both the medicaments showed a significant reduction of *S. mutans* and *L. acidophilus* bacterial counts ($p < 0.001$) after one week of treatment. Statistically, there was no significant difference in the individual average reduction ($p = 0.820$ and 0.277) as well as combined mean reduction ($p = 0.314$) of *S. mutans* and *L. acidophilus* bacterial counts among the groups. This indicated that both the medicaments possessed comparable overall antibacterial effects.

Additionally, in this study, Formocresol exhibited a higher antibacterial effect against *S. mutans* than *L. acidophilus*. *Allium sativum* oil exhibited a higher antibacterial effect against *L. acidophilus* than *S. mutans*. This finding adds up to the effectiveness of herbal medicament in irreversibly inflamed/ necrotic primary teeth.

In the present study, the antibacterial effect of Formocresol was strong, especially against *S. mutans* and *L. acidophilus*. This finding is concomitant with other studies that have found Formocresol to have strong antibacterial and fixative properties.^{18,19} Moreover, the overall antibacterial effect of Formocresol was higher compared to *Allium sativum* oil in the present study. However, statistically, the difference was insignificant, contrary to another similar study in which non-vital pulpotomy procedure was performed in primary non-vital teeth, followed by bacteriological evaluation was done.⁷

Significant decrease in salivary *S. mutans* bacterial count was reported by *A. sativum* extract in a systematic review and meta-analysis as a mouthwash. Although this study was on *Allium sativum* extract, but resulted in potent antibacterial effect, similar to the present study. The reason might be due to the similar mechanism of action of Garlic. Allicin present in Garlic is responsible for the antibacterial effect by inhibiting thiol-containing enzymes responsible for nutrition of bacteria.²⁰

In the present study, *Allium sativum* oil showed better clinical outcomes compared to Formocresol as a non-vital pulpotomy medicament in primary molar teeth. However, statistically, the difference was insignificant i.e. $p > 0.999$. Similar to the present study, clinical outcomes of medicaments with statistically insignificant differences have been reported in several other studies in which Formocresol was used as a pulpotomy medicament compared with *Allium sativum* extract,²¹ *Allium sativum* oil,²² *Aloe barbadensis* gel, and *Allium sativum* oil,²³ and extracts of *Miswak*, *Allium sativum*, and *Nigella sativa*.¹⁴

However, contrary to the present research in which both the medicaments showed comparable clinical results, in a previous

study *Allium sativum* oil exhibited significantly better clinical as well as radiographic results than that of Formocresol as non-vital pulpotomy medicament.¹

In a histological evaluation, *Allium sativum* oil compared with Formocresol in permanent teeth,²⁴ and with tri-antibiotic paste and Formocresol in primary teeth²⁵ showed significantly better dentine bridge formation and healing potential. The results could not be compared to the current study as those were histological evaluations. However, the findings of these studies further support the potential use of *Allium sativum* oil as pulpotomy medicament.

The limitations of the study were the small sample size and short clinical follow-up period that may have effect on the study results. Furthermore, longer and variable sample transportation time might have influence on the viability of bacterial colonies.

CONCLUSION

In this study, the antibacterial effect of *Allium sativum* oil was evaluated and compared with Formocresol, against *S. mutans* and *L. acidophilus*, in terms of bacterial count (CFU/ml) in non-vital pulpotomy of primary molars. The results indicated comparable antibacterial effect. Additionally, the clinical outcomes of both medicaments were also comparable supporting the reliability of non-vital pulpotomy procedure itself. This concludes that *Allium sativum* oil can be used as an effective alternative to Formocresol in non-vital pulpotomy of primary molars.

DISCLOSURE:

This is an article derived from the thesis.

ETHICAL APPROVAL:

Ethical approval was obtained from Ethical Review Committee, Post-Graduate Medical Institute/ Ameer-ud-Din Medical College/ Lahore General Hospital, Lahore (AMC/ PGMI/ LGH/ Synopsis No./0081-19).

PATIENTS' CONSENT:

Informed consent was obtained from patients' parents.

COMPETING INTEREST:

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

MM: Design investigations of work and write up.

SN: Conception, critical analysis, and data analysis and interpretation.

AZ, NB, AH: Drafting the manuscript.

All authors approved the final version of the manuscript to be published.

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