# **ORIGINAL ARTICLE**

# Turmeric gel as an alternative non-vital pulpotomy medicament in primary teeth- a randomized controlled trial

Mashal Mazhar<sup>1\*</sup>, Shazia Naz<sup>2</sup>, Ambreen Zahra<sup>3</sup>, Nazia Bashir<sup>4</sup>, Rabia Dastgeer<sup>5</sup>

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# ABSTRACT

**Background and Objective:** Formocresol is a gold standard medicament in both vital and non-vital pulpotomies due to its potent antibacterial potential but, it has a controversial nature due to its side effects. Therefore, the demand for natural alternatives of Formocresol as a pulp dressing material, with few or no side effects, became imperative. The purpose of the study was to introduce turmeric gel as a non-vital pulpotomy (NVP) medicament in primary teeth by evaluating its antibacterial effect against *Streptococcus mutans* and *Lactobacillus acidophilus*.

**Methods:** It was a multiarm parallel randomized controlled trial in which sixty patients aged between 4 and 8 years, each containing at least one non-vital primary molar, were selected and randomly divided into group A (Formocresol) and group B (Turmeric gel). 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 1 week. Samples were again taken, and the tooth was restored. A comparison between bacterial count at baseline and after 1 week of treatment was made and expressed as Colony-Forming Units/ml. Data were entered and analyzed by using Statistical Package for Social Sciences version 25.0.

**Results:** There was a significant reduction in median *S. mutans* and *L. acidophilus* bacterial count in each group after 1 week of treatment (p < 0.001). Formocresol showed a higher average reduction than turmeric gel. However, statistically, the difference was insignificant indicating that both the medicaments possessed comparable antibacterial effects.

Conclusion: Turmeric gel was found as an effective alternative to Formocresol for its use as a non-vital pulpotomy medicament.

Keywords: Formocresol, turmeric (curcuma), pulpotomy, non-vital, primary (deciduous) teeth, randomized controlled trial.

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 Correspondence to:
 Mashal Mazhar
 \*
 \*
 Senior Demonstrator, Department of Operative Dentistry, University College of Dentistry, The University of Lahore, Lahore, Pakistan.

 Email:
 mashalmazhar91@gmail.com
 \*

Full list of author information is available at the end of the article.

# Introduction

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

Bacteria present in primary teeth with necrosed pulp are poly-microbial, predominantly *Streptococcus mutans* (*S. mutans*) and *Lactobacillus acidophilus* (*L. acidophilus*).<sup>4</sup> *Streptococcus mutans* is commonly regarded as the dental caries initiator,<sup>5</sup> and *L. acidophilus* as the dental caries progressor.<sup>6</sup>

Treatment of choice to save cariously exposed non-vital yet restorable primary teeth is pulpectomy, as the damaged pulp is unable to heal itself.<sup>7</sup> Despite its high success rate, pulpectomy is often impractical in some pediatric patients such as, when there is difficulty 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's cooperation.<sup>1,7</sup> Extraction is another option for the

management of non-vital primary teeth, where the tooth is non-restorable due to extensive caries and severe mobility.<sup>8</sup>

Non-vital pulpotomy (NVP) also known as mortal pulpotomy, is regarded as a quick, least invasive alternative with predictable outcomes for the decayed non-vital, yet restorable primary teeth. NVP is performed when neither pulpectomy nor extraction is feasible. In NVP, the infected non-vital radicular pulp is dressed with a strong antiseptic material that combats infection, mummify, fix, and sterile the necrotic pulp tissue so that it can stay *in situ* till the tooth exfoliates.<sup>9</sup>

Several conventional materials have been used in pulpotomy, commonly mineral trioxide aggregate,<sup>10</sup> 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 effects<sup>11</sup> such as mutagenicity, carcinogenicity, allergenicity, systemic distribution,<sup>12</sup> tissue changes in internal organs, and enamel defects in permanent successors.<sup>1</sup> Due to the controversial nature of Formocresol, it seemed imperative to do research to find clearly and reproducibly safer, superior alternatives to Formocresol.<sup>11</sup>

Despite the advancements in modern medicine, a viable health alternative for large under-developed and underprivileged sections of the world continues to be traditional medicine.<sup>13</sup> In dentistry, several herbal medicaments have been used in pulpotomy procedures in pediatric patients<sup>14</sup> 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.<sup>15</sup>

Turmeric (*Curcuma longa*) possesses potent antibacterial potential,<sup>16</sup> being anti-inflammatory, analgesic, antioxidant, and anti-cariogenic.<sup>17</sup> Turmeric has only been used in vital pulpotomy.<sup>18,19</sup>

There is a lack of international literature, and to the authors' best knowledge, no evidence of local study has been found on the NVP procedure and medicaments used in this procedure.

There was a need for further analysis, research, and randomized controlled trials (RCTs) on turmeric to promote its usage in dental practice as an alternative to Formocresol and the applicability of the NVP procedure itself in local patients.

The study aimed to find a suitable herbal alternative (Turmeric gel), to replace the routinely used conventional medicament, Formocresol, in the NVP procedure with a hypothesis that there was no difference in the antibacterial effect of turmeric gel compared with gold standard Formocresol, against *S. mutans* and *L. acidophilus*, in NVP

of primary molars in terms of average reduction of bacterial count (Colony-Forming Units/ml - CFU/ml).

# **Methods**

It was a double-blinded, randomized controlled trial (Fig:1) The sample size in each group was 20. The study was conducted at the Pediatric Dentistry Department, Punjab Dental Hospital, Lahore, Pakistan, in collaboration with the Microbiology Department of Lahore General Hospital, Lahore. The study was done from October 2022 to February 2023. Forty cariously exposed non-vital primary molars from forty pediatric patients (one tooth from each patient) of both sexes aged between 4 and 8 years were selected from the Outpatient Department of Pedodontics, de'Montmorency College/Punjab Dental Hospital, Lahore. Ethical approval was obtained from the Institutional Ethical Review Committee and consent from the parents of the patients was taken before enrollment of the study participants.

The patients were randomly allocated into two groups using the lottery method. The participants were blinded to which medicament group they belonged.

Group A = Formocresol (control group, standard medicament) (n = 20)

Group B = Turmeric gel (n = 20)

# Formocresol

Premade Formocresol (Tricresol & Formalin) was used composed of Tricresol 35%, Formaldehyde (40%) 19%, excipient additive 100% (PD, Switzerland, ISO9001/ISO9001\_2000/ISO13485/CE\_MARK), (Universal Dental Pvt, Ltd).

#### Turmeric gel

Turmeric gel was self-prepared at the Institute of Microbiology and Molecular Genetics, Punjab University, Lahore, Pakistan. A total of 2 kgs of turmeric rhizomes were purchased from a local market in Lahore and verified by a taxonomist, Botany Department, Government College University, Lahore, Pakistan (voucher number: GC.Herb.Bot.3693). The rhizomes were then washed, cut, and sun-dried for 3 days then oven-dried at 60°C for 30 minutes, and then grounded into powdered form. Turmeric powder (180 g) was obtained which was then transferred into sterilization pouches and autoclaved at 121°C for 30 minutes. Approximately 170 g powder along with 550 ml distilled water was taken in a Soxhlet extractor. After 96 hours, ~30% aqueous turmeric extract was collected and filtered repeatedly through the Whatman No.1 filter paper. The obtained filtrate (Turmeric extract) was then mixed with 6% Sodium Carboxy-Methyl Cellulose (NaCMC) (Genevex Chem, Hyderabad, India) to form a gel. Four Vitamin C grounded tablets (500 mg of ascorbic acid per tablet) and 12 Vitamin E capsules (200 IU per capsule) (Evion, Martin Dow Pharmaceuticals, Ltd. Karachi, Pak) then added in 100 g of gel as antioxidants. The prepared gel had 21 days shelf-life.<sup>20</sup>

# Preparation of cavity and NVP

For ease of procedure, local anesthesia 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 (Meisinger, Germany) using a high-speed handpiece with constant water spray. The pulp chamber was accessed, and de-roofing was done using non-end cutting steel straight fissure bur (Meisinger, Germany). Removal of the coronal pulp tissue (if any) was achieved with a sterile, sharp spoon excavator type-A (Humayun Dental Supplies, Pakistan).<sup>7</sup>

# Sample collection

The samples were collected for *in-vitro* evaluation. One sample was taken from each root canal by dipping #20 sterile paper points 2-3 mm into the root canal for 10 seconds. These paper points were then immediately placed into plastic calibrated screw-capped tubes containing 5 ml of autoclaved Fluid Thioglycollate Transport Medium (CM0173 Oxoid, UK),<sup>21</sup> labeled with laboratory code and transported to the laboratory as soon as possible.<sup>7,22</sup>

# Placement of dressings

For group A (Formocresol), the radicular pulp was dressed with #4 (3 mm) cotton pellet which was dipped in respective medicament. For group B, approximately 3-4 mm of turmeric gel matching the size of #4 cotton pellets was placed over root canals' orifices with plastic instrument type-A. The tooth was then restored temporarily with a thin layer of Cavit (Detax, Germany), followed by a thick hard mix of zinc oxide and eugenol type II (Shanghai New Century Dental Materials Co., Ltd). The patient was then recalled after 1 week. Again, sample collection was done and transported to the laboratory. In addition, the patient was clinically assessed according to criteria by Mohammad and Baroudi<sup>7</sup>.

### Processing and culturing of bacterial samples

Blood agar (CM0055 Oxoid, UK) was used for culturing *S. mutans* and de Man, Rogosa, Sharpe (MRS) agar (CM0361 Oxoid, UK) 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. 1  $\mu$ l portion of the sample was taken with 1  $\mu$ l disposable plastic loop (Oxoid, UK) and spread on respective culture media plates. The inoculated plates were then placed in an anaerobic jar with a gas pack (AN0025A Oxoid, UK) and incubated for 2 days (48 hours) at 37°C.<sup>7</sup>

# Streptococcus mutans and L. acidophilus identification

Bacterial colonies were identified by the naked eye, Gramstaining, and catalase test. Further identification was done under a light microscope (Olympus CH-2) with 100× magnification.

#### Rapid bacterial identification kits and software

For species level identification, Analytical Profile Index kit (API 20 STREP kit, bioMérieux Ref 20 690), and API 50 CHL kit (bioMérieux Ref 50 410 and 50 300) 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* (bioMérieux apiweb<sup>TM</sup> identification software - Ref 40 011) for interpretation.

#### Counting of bacterial colonies

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

# Statistical analysis

Data were entered in Statistical Package for Social Sciences<sup>\*</sup> version 25. 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 to compare the median reduction of bacterial count between the groups. Fisher's exact test was used to compare clinical outcomes among different groups. A  $p \le 0.05$  was considered as significant.

#### Results

Bacterial colonies of the *S. mutans* appeared grayish-white, smooth, glossy, and translucent with zones of alpha hemolysis (partial hemolysis with greenish-brown discoloration) on blood agar plates (Figure 2a). They were Gram-positive, catalase negative, and appeared cocci-shaped, present in pairs and chains, without spores under a light microscope (Figure 2b). Bacterial colonies of *L. acidophilus* appeared compact, smooth, medium, opaque, and white mucoid on the MRS agar plate (Figure 3a). They were Gram-positive, catalase catalase-negative and appeared as round-ended single, pairs, and chains of rods without spores under a light microscope (Figure 3b). Further biochemical testing using the API 20 STREP kit for *S. mutans* and API 50 CHL kit for *L. acidophilus* was done, and the results were analyzed through API software.

The data were not normally distributed according to Shapiro-Wilk test so the median bacterial count for both groups was calculated. Results of the Mann-Whitney *U* test showed statistically no significant difference in the

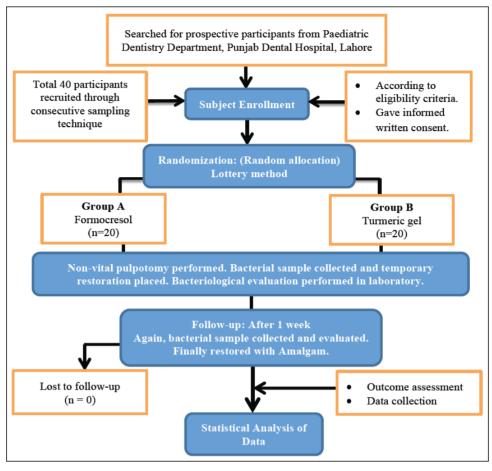


Figure 1. Study flow chart according to CONSORT guidelines.

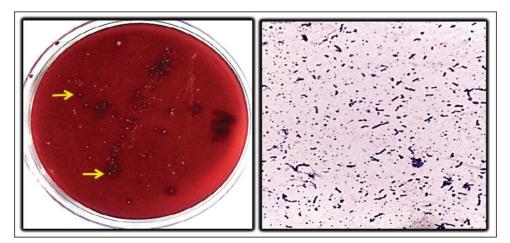


Figure 2. (a): Streptococcus mutans colonies on blood agar plate, (b): Microscopic view of S. mutans.

average reduction of *S. mutans* bacterial count as well as *L. acidophilus* bacterial count among the groups. For analysis of the combined bacterial count among the groups, the data were normally distributed so the mean bacterial count for both the groups was calculated. Results of Mann-Whitney *U* test showed statistically no significant difference in the mean

reduction of combined bacterial count among the groups (p = 0.640) (Table 1) indicating that the overall antibacterial effect of both the medicaments was similar. Clinical outcome was analyzed through Fisher's exact test which revealed that there was a statistically insignificant difference in the clinical outcome among the groups (p = 0.487) (Table 2).

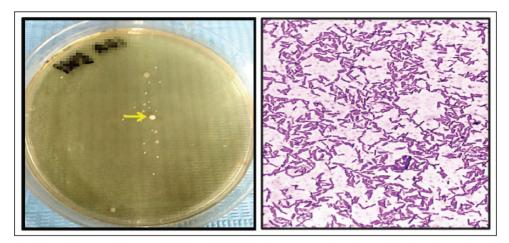


Figure 3. (a): Lactobacillus acidophilus on MRS agar plate, (b): Microscopic view of L. acidophilus.

 Table 1. Comparison of mean reduction of combined bacterial count among group.

Group	Mean reduction of overall bacterial count (CFU/ml)				
	Mean ± SD	Minimum	Maximum	<i>p</i> -value <sup>#</sup>	
Group A	30,300 ± 14,060	13,000	60,000	0.640	
Group B	29,300 ± 7,399	14,000	44,000		

Group A: Formocresol, Group B: Turmeric gel, #Mann-Whitney U test.

# Discussion

The study was designed to evaluate the antibacterial effect of Formocresol (Group A), and turmeric gel (Group B) against *S. mutans* and *L. acidophilus* in pediatric NVP as 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 as the antibacterial effect of each medicament.

Non-vital (Mortal) pulpotomy procedure was opted for in this study as it is often used as a quick, least invasive alternative for the decayed, irreversibly inflamed/nonvital, yet restorable primary teeth. It is performed when neither pulpectomy nor extraction is feasible.<sup>9</sup> Pulpectomy is impractical in some pediatric patients with inadequate access, limited cooperation, and possible damage to permanent tooth germ.<sup>1,7</sup> Extraction is another option but is an invasive procedure and can lead to space maintenance issues.<sup>23</sup>

The most significant finding of the present study was that both the medicaments showed a significant reduction of *S*. *mutans* and *L*. *acidophilus* bacterial count after 1 week of treatment. Statistically, there was no significant difference in the individual average reduction (p = 0.718 and 0.968) as well as combined mean reduction (p = 0.640) of *S*. *mutans* and *L*. *acidophilus* bacterial count among the groups. This indicated **Table 2.** Comparison between the effects of all three medicaments on the clinical outcome of non-vital primary teeth after 1 week (1st follow-up visit).

Clinical findings after 1 week	Group A n = 20 (%)	Group B n = 20 (%)	<i>p</i> -value*
Post-operative pain	(+) 2 (10.0%)	(-)	0.487
Tenderness to percussion	(-)	(-)	
Gingival inflammation	(-)	(-)	
Draining sinus	(-)	(-)	
Increased mobility (Grade II and III)	(-)	(-)	

Group A: Formocresol, Group B: Turmeric gel, \*Fisher's exact test + (having signs and symptoms), - (without signs and symptoms).

that both the medicaments possessed comparable overall antibacterial effects.

In addition, in this study, Formocresol exhibited a higher antibacterial effect against *S. mutans* than *L. acidophilus*. Turmeric gel exhibited an equal antibacterial effect against both *S. mutans* and *L. acidophilus*. 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 where Formocresol exhibited potent antibacterial and fixative properties,<sup>24,25</sup> but as an intracanal medicament on extracted primary canines.

In dentistry, turmeric is famous for its inhibitory potential specifically against caries-causing bacteria.<sup>26</sup> In the present study, the antibacterial potential of aqueous turmeric gel made from turmeric rhizomes has been concluded to be potent, especially against *S. mutans* and *L. acidophilus*. This finding is similar to other studies, even when combined with

photodynamic therapy,<sup>27</sup> as well as when compared with *Aloe vera* and Nutmeg<sup>28</sup> and with Chlorhexidine.<sup>29</sup>

The antibacterial effect of methanol Curcumin extracts against *S. mutans* and *Streptococcus pyogenes*,<sup>30</sup> and aqueous Curcumin extract against *S. mutans* as well as *Staphylococcus aureus*, and anaerobic bacteria<sup>31</sup> has been reported in the literature.

The similarity in the antibacterial finding is because of constituents of turmeric i.e Curcuminoids mainly Curcumin, bisdemethoxycurcumin, and demethoxycurcumin, which are responsible for the antibacterial nature of turmeric leading to disruption of the cell wall and ultimately death of the specific bacteria.<sup>32</sup>

However, turmeric leaf extract exhibited a potent antibacterial effect against a wide range of bacteria but not against *L. acidophilus* which is not concomitant with the present study. This might be due to the use of leaf extract of turmeric which allegedly contains oligosaccharide compounds. These oligosaccharides provide media for the growth of *Lactobacillus* instead of its inhibition.<sup>33</sup>

In the present study, turmeric gel showed better clinical outcomes compared to Formocresol as an NVP medicament in primary molar teeth of pediatric patients. However, statistically, the difference was insignificant among the two, i.e., *p*-value = 0.487 (Table 2). This finding is supported by a study in which turmeric gel showed clinical success as a vital pulpotomy medicament comparable to Formocresol, Propolis, and Calcium Hydroxide.<sup>18</sup> Turmeric powder also showed significantly promising clinical results in pulpotomy of primary molars.<sup>19</sup> Although the above studies were carried out on vital teeth, the results were concomitant with the present study.

# Conclusion

The antibacterial effect and clinical outcomes of turmeric gel are comparable to the conventional medicament, Formocresol, against *S. mutans* and *L. acidophilus*, in terms of bacterial count (CFU/ml) in NVP of primary molars of pediatric patients. Therefore, turmeric gel can be used as a safe, cost-effective herbal alternative to Formocresol in the NVP of primary molars.

#### Limitations of the study

The limitations of the study were the small sample size and short clinical follow-up period that may have an effect on the generalizability of the study results. Furthermore, longer and variable sample transportation time might have an influence on the viability of bacterial colonies which can be standardized by adopting more sensitive techniques for determination of colony counts.

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#### **List of Abbreviations**

AMC	Ameer Uddin Medical College
CFU/ml	Colony Forming Units/ml
LGH	Lahore General Hospital
NaCMC	Sodium Carboxy-Methyl Cellulose
NVP	Non-vital pulpotomy
PGMI	Postgraduate Medical Institute
SPSS	Statistical Package for Social Sciences

#### **Conflict of interest**

None to declare.

**Grant support and financial disclosure** None to disclose.

#### **Ethical consideration**

Ethical approval was obtained from the Institutional Ethical Review Committee of Postgraduate Medical Institute; Lahore Pakistan vide Letter No: AMC/ PGMI/ LGH/ Synopsis No./ 0081-19 dated 19-04-2019. This is an article derived from the thesis of Masters of Dental Surgery, which has been recently approved for award of degree (Ref # No: UHS/CE-23/T-Exam/6329).

#### **Clinical trial registration**

The study was registered as a RCTs in ClinicalTrials.gov: NCT05801354 dated April 6, 2023.

#### **Authors' contributions**

AZ, NB, RD: Drafting of the manuscript, acquisition of data
MM: Principal investigator, concept and design of study
SN: Supervisor, conception of study, critical intellectual input, data analysis and interpretation.

**ALL AUTHORS:** Approval of the final version of the manuscript to be published.

#### **Authors' Details**

Mashal Mazhar<sup>1</sup>, Shazia Naz<sup>2</sup>, Ambreen Zahra<sup>3</sup>, Nazia Bashir<sup>4</sup>, Rabia Dastgeer<sup>5</sup>

- 1. Senior Demonstrator, Department of Operative Dentistry, University College of Dentistry, The University of Lahore, Lahore, Pakistan
- Associate Professor, Department of Operative Dentistry, de' Montmorency College of Dentistry, Lahore, Pakistan
- 3. Demonstrator, Department of Operative Dentistry, Avicenna Medical and Dental College, Lahore, Pakistan
- 4. Dental Surgeon, Jinnah Burn and Reconstructive Surgery Center, Jinnah Hospital, Lahore, Pakistan
- 5. Senior Demonstrator, Department of Dentistry, Al Aleem Medical College, Lahore, Pakistan

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