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Dentistry Section

# Evaluation of Antimicrobial Efficacy of 3.8% Silver Diamine Fluoride as a Root Canal Irrigant against *Enterococcus Faecalis* in Primary Teeth: A Randomised Clinical Trial

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## ABSTRACT

**Introduction:** During a pulpectomy, the infected or inflamed pulp tissues are removed, and the root canal is thoroughly cleaned with mechanical instrumentation and copious irrigation. Various endodontic irrigants are available, such as Sodium Hypochlorite (NaOCl), Chlorhexidine (CHX) gluconate, Ethylene Diamine Tetra Acetic Acid (EDTA), Mixture of doxycycline, citric acid, and a detergent (MTAD), etc. Among these, CHX gluconate is widely used as an endodontic irrigant and medicament due to its antibacterial effect and substantivity. Enterococcus faecalis is the most common bacterial species found in necrotic teeth, with high viability and antibiotic resistance. A 3.8% Silver Diamine Fluoride (SDF) has antimicrobial properties and has been shown to eliminate *E. faecalis* from the root canals of permanent teeth. However, there are no studies in the literature that have evaluated its efficacy as a root canal irrigant in primary teeth.

**Aim:** To evaluate the antimicrobial efficacy of 3.8% SDF against *E. faecalis* in primary teeth.

**Materials and Methods:** This was a in-vivo double-blinded randomised clinical trial conducted at Department of Paediatric and Preventive Dentistry, GSL Dental College and Hospital, Rajahmundry, Andhra Pradesh, India in children aged 3-8 years old. The study included 60 primary teeth that required pulpectomy. The teeth were divided into two groups and irrigated: 30 teeth with 3.8% SDF (Group I) and 30 teeth with 2% CHX (Group II). In all cases, two microbiological samples were taken using sterile absorbent paper points: the first after access opening and the second after the final irrigation. All samples were assessed using the agar plate method. The results were analysed statistically using a Student's paired t-test.

**Results:** After analysing the pre- and postirrigation samples, there was a statistically significant reduction in Colony Forming Units (CFU)/mL ($p<0.05$) in both groups. When comparing the two groups, no statistical difference was observed in the percentage reduction of bacterial colonies ($p>0.05$).

**Conclusion:** The reduction in CFU/mL of *E. faecalis* was comparable in both groups. Thus, 3.8% SDF can be used as an alternative root canal irrigant to 2% CHX.


**Keywords:** Antimicrobial agents, Chlorhexidine, Endodontics, Pulpectomy, Therapeutic

## INTRODUCTION

Primary teeth play a significant role in the growth and development of a child's dentofacial structure, serving various functions such as chewing, speech, and the development of occlusion, until the eruption of permanent teeth. However, multifactorial diseases like dental caries can progress to pulpitis [1]. Primary teeth are more vascular and cellular than permanent teeth, which accelerates the development of pulpal diseases. Dental caries can cause malocclusion, loss of arch length, disruption of dentofacial growth, and even early tooth loss. Therefore, it is crucial to maintain good oral hygiene practices and schedule regular dental check-ups for children to prevent and treat dental caries, which can have long-term consequences on their overall oral health and development.

Additionally, early intervention and treatment of dental caries in primary teeth can prevent the need for more invasive and costly treatments in the future [2]. The preservation of carious teeth and the promotion of normal growth and development are the ultimate goals of paediatric dentists with regard to a child's well-being. Thus, endodontic treatment is necessary to address affected teeth [1]. A pulpectomy is the preferred treatment option since alternative pulp therapies have a lower success rate in preserving primary teeth until their natural exfoliation time. The pulpectomy procedure involves three steps: access opening, cleaning and shaping, and obturation [3].

Microorganisms are a major cause of pulpal and periapical diseases. It is challenging to eliminate microbes from infected root canals, and *Enterococcus faecalis*, a gram-positive facultative anaerobe, is frequently found in root canals, particularly in secondary endodontic infections [3]. *E. faecalis* demonstrates virulence and the ability to survive in highly acidic and alkaline conditions. Various instrumentation techniques, irrigation protocols, and intracanal medications are employed to eradicate microorganisms. No single method alone can completely eliminate bacteria in root canals. Among the different methods used, irrigation plays a significant role [4].

Numerous irrigants are available, but to date, none of them are perfect, and each has its drawbacks. Sodium Hypochlorite (NaOCl), Chlorhexidine (CHX), Ethylenediaminetetraacetic acid (EDTA), Citric Acid (CA), a combination of tetracycline isomers, acid, detergent (MTAD), and Hydrogen Peroxide ($H_2O_2$) are commonly used irrigants that have proven effective against *E. faecalis* [4]. However, none of them are entirely effective in completely elimination bacteria. Silver Diamine Fluoride (SDF) with its silver and fluoride content has shown greater efficacy in completely elimination *E. faecalis* in permanent teeth. However, its use as a root canal irrigant in primary teeth has been less evaluated [5]. The present study aimed to evaluate the efficacy of 3.8% SDF in primary root canals, as it has been used in endodontic medicaments.

## MATERIALS AND METHODS

The present study was an in-vivo double-blinded randomised clinical trial conducted at Department of Paediatric and Preventive

Dentistry, GSL Dental College and Hospital, Rajahmundry, Andhra Pradesh, India on children aged 3-8 years who visited the Outpatient Department of paediatric and preventive dentistry. The study included patients who reported between April 2021 and March 2022. The study received approval from the Institutional Ethical Committee (IEC Ref No: GSLDC/IEC/2021/013) and was registered in the Clinical Trials Registry-India database (CTRI/2021/09/036881). The purpose of the study was explained to the parents or guardians, and written informed consent was obtained from them. A total of 60 teeth requiring pulpectomy were included in the study.

**Inclusion criteria [6]:**

- Patients in good general health.
- Primary teeth (anterior/posterior) with atleast one necrotic pulp canal, abscess, or sinus tract.
- Presence of radiolucent area in the furcation or periapical region.
- Atleast two-thirds of the root remaining.
- Sufficient tooth structure to support a rubber dam.
- Adequate isolation and sterility control in the operative field to prevent bacterial growth.

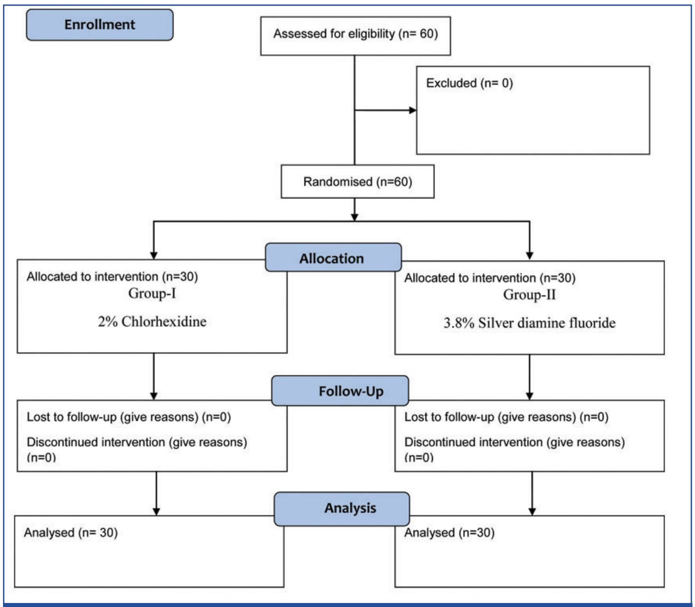
**Exclusion criteria [6]:**

- Patients who had received antibiotics within two weeks prior to sampling or those with any systemic diseases.
- Patients with non restorable teeth, perforated pulpal floor, excessive mobility, or pathological root resorption.

**Sample size calculation:** A sample of 60 was determined using power calculation based on published studies [6], resulting in 80.0% power and 5% Type-I error probability (α=0.05%). The samples were randomly divided into two groups.

Group I (n=30): 2% Chlorhexidine gluconate (control).

Group II (n=30): 3.8% Silver Diamine Fluoride (SDF) (experimental) [Table/Fig-1].




**[Table/Fig-1]:** Consolidated Standards of Reporting Trials (CONSORT) flowchart.

## Study Procedure

All procedures were completed in a single appointment, and periapical radiographs of the selected teeth were taken. About 2% lidocaine was used for inferior alveolar nerve block in primary mandibular teeth and infiltration (palatal and buccal) for primary maxillary teeth after oral cavity antisepsis. After the access opening, the first microbiological sample was taken from inside the canal (pre-irrigation) using a sterile absorbent paper point of size no. 15 (2% taper) matching the root canal diameter, which was kept in place for 30 seconds. The extracted paper points were immediately placed in a test tube containing Himedia Brain Heart Infusion (BHI) broth [7], which served as a transport and growth medium to keep the sampled bacteria alive. Following sample collection, all teeth underwent instrumentation with 2% taper K-files and were irrigated with 0.5 mL of the chosen solution at each filing. The canal was irrigated for the final time after instrumentation and before obturation [8].

At that moment, a second microbiological sample was collected from the same canal using a size no. 20 paper point (2% taper) for 30 seconds, and the retrieved paper point was immediately placed into a BHI broth test tube. The canal was then obturated using Zinc Oxide and Eugenol (ZOE) or metapex obturating material, and an intraoral periapical radiograph was taken after the procedure. The collected samples were subjected to microbiological analysis to determine the number of Colony-Forming Units (CFU) of *Enterococcus faecalis*.

**Laboratory procedures:** The pre- and postirrigation samples were streaked out on petri plates and placed in an anaerobic gas jar for 48 hours. Once bacterial growth was observed, the magenta-pink-coloured colonies were inoculated on a slide, and gram staining was performed. The bacterial colony forming units were counted in the inoculated samples under a microscope using the turbidimetry method and McFarland's scale pattern. This method calculates the number of bacteria in suspension (as CFU/mL) by comparing the different values of turbidity or density on the scale [Table/Fig-2-4] [6].


**[Table/Fig-2]:** Showing counting bacterial colonies on the digital colony counter.

**[Table/Fig-3]:** Showing bacterial colonies of Group I.

[Table/Fig-4]: Showing bacterial colonies of Group.

## STATISTICAL ANALYSIS

The obtained data was tabulated and subjected to statistical analysis. Descriptive statistics, independent samples t-tests, and paired t-tests were performed to analyse the study data. The data was analysed using Statistical Package for Social Sciences (SPSS) software with a significance level of 0.05.

## RESULTS

The mean CFU count at baseline in the 3.8% SDF group was 56333.33±21412.7, and after instrumentation and irrigation, it was 18666.67±8193.07 [Table/Fig-1]. In the 2% CHX group, the mean CFU counts at baseline and post-instrumentation were 73333.33±15829.55 and 40333.33±14015.59, respectively [Table/Fig-5].

There was a significant difference (p=0.001) in the mean CFU count at baseline between the two study groups, with significantly higher mean values in the 2% CHX group. Similar observations were noted after instrumentation and irrigation, with higher mean CFU counts in the 2% CHX group (40333.33±14015.59) compared to the 3.8% SDF group (18666.67±8193.07).

Within each of the two study groups, there was a significant decline (p<0.001) in the mean CFU counts from baseline to post-instrumentation [Table/Fig-6].

However, when the mean change from baseline to post-instrumentation was calculated for each sample and compared between the study groups, there was no significant difference (p=0.194) between the 3.8% SDF group (37666.67±15905.61) and the 2% CHX group (33000.00±11188.04). There was a 45% drop in colony forming units (CFU/mL) from baseline to post-instrumentation cultures in the control group and a 66.8% decrease in CFU counts from baseline to post-instrumentation samples in the experimental group.

## DISCUSSION

The main issues with using root canal irrigants are their inability to reach the apical third and inaccessible areas (such as lateral and accessory canals, isthmus), their clinical usage time, and their toxicity to periapical tissues. Additionally, their effectiveness is greatly influenced by the presence of infected debris (organic and inorganic) [4].

It has been well-established in the literature that chlorhexidine gluconate (CHX), an antibacterial solution, can be useful in endodontic therapy. CHX has been used for general disinfection purposes and for the treatment of skin, eye, and throat infections in both humans and animals. It is a synthetic cationic bis-guanide with two symmetric 4-chlorophenyl rings, two bis-guanide groups, and two central hexamethylene chains. This molecule easily dissolves in water and is stable as a salt [9].

| Time | Parameter | | Statistics of 3.8% SDF | Statistics of 2% CHX | p-value |
|---|---|---|---|---|---|
| Baseline | Mean | | 56333.33 | 73333.33 | 0.001 |
| | 95% Confidence interval for mean | Lower bound | 67422.48 | 48337.68 | |
| | | Upper bound | 79244.19 | 64328.98 | |
| | Median | | 55000.00 | 75000.00 | |
| | Std. deviation | | 21412.747 | 15829.552 | |
| | Minimum | | 30000 | 40000 | |
| | Maximum | | 90000 | 100000 | |
| | Range | | 60000 | 60000 | |
| Post-instrumentation | Mean | | 18666.67 | 40333.33 | 0.001 |
| | 95% Confidence interval for mean | Lower bound | 35099.83 | 15607.32 | |
| | | Upper bound | 45566.84 | 21726.01 | |
| | Median | | 20000.00 | 40000.00 | |
| | Std. Deviation | | 8193.072 | 14015.591 | |
| | Minimum | | 10000 | 10000 | |
| | Maximum | | 30000 | 80000 | |
| | Range | | 20000 | 70000 | |

[Table/Fig-5]: Showing mean CFU counts of both the groups at baseline and post-instrumentation.

| Group | Time | Mean | N | Std. Deviation | Std. Error mean | t value | p-value |
|---|---|---|---|---|---|---|---|
| 3.8% SDF | Baseline | 56333.33 | 30 | 21412.747 | 3909.415 | 12.97 | <0.001* |
| | Post-instrumentation | 18666.67 | 30 | 8193.072 | 1495.844 | | |
| 2% CHX | Baseline | 73333.33 | 30 | 15829.552 | 2890.068 | 16.15 | <0.001* |
| | Post-instrumentation | 40333.33 | 30 | 14015.591 | 2558.885 | | |

[Table/Fig-6]: Comparison of change in colony forming units from baseline to post-instrumentation in each of the two study groups.
Paired t-test; p≤0.05 considered statistically significant; *denotes statistical significance

The CHX, a broad-spectrum antibacterial, is effective against yeast, gram-positive and gram negative bacteria, and other microorganisms. Its cationic molecular component binds to areas of negatively charged cell membranes, causing lysis of the cells. CHX has been used for many years as a mouthwash and periodontal irrigant in periodontal therapy, implantology, and cariology to control dental plaque [10].

A 2% concentration of CHX is suggested as the final rinse irrigant due to its substantivity, which allows it to bind to dentin and provide persistent antibacterial action, particularly in endodontic retreatment. The CHX molecule can attach to proteins like albumin found in serum or saliva, the pellicle on the surface of the tooth, salivary glycoproteins, and mucous membranes due to its cationic properties. This attachment is reversible [11]. Additionally, it can adhere to hydroxyapatite and teeth. This reversible uptake and release of CHX, known as substantivity, is dependent on the concentration of CHX and results in significant antibacterial activity [12].

Numerous studies have found that CHX is more effective at killing bacteria than other irrigants [6,9,10,13]. According to Ercan E et al., the antibacterial activity of 2% CHX was higher than that of 5.25% sodium hypochlorite (NaOCl) [14]. The type, concentration, and presentation form of the irrigants, as well as the susceptibility of the microorganisms, can affect the antibacterial effect of CHX. CHX is effective against bacteria but has no effect on biofilm or other organic waste. A 2% concentration of CHX may be a good option for optimal antibacterial activity [15].

In the study, it was observed that there was a 45% decrease in Colony-Forming Units (CFU/mL) from baseline to post-instrumentation cultures in the CHX group. Similar findings were reported by another author who found that CHX was a superior antibacterial agent against both endodontic aerobes and anaerobic microbes in primary teeth. Pre- and postirrigation samples in the 2% CHX group showed a decrease in CFU/mL [16]. However, contradictorily, another study found that 2% CHX was not as successful as other root canal irrigants in removing root canal bacteria. Additionally, CHX lacks the ability to dissolve tissue [17].

In current research, SDF is being used as an experimental irrigant against CHX. SDF is a colourless solution that can be used for tooth remineralisation and is available in concentrations of 3.8% to 38%. The 3.8% preparation was specifically developed for root canal therapy [18].

While various antibacterial treatments have been used to disinfect root canals, there have been reports of *Enterococcus faecalis* resistance. Traditionally, an ammoniated silver nitrate solution has been used to treat root canal infections. However, the application of SDF solution as a root canal irrigant has shown a significant decrease in the number of needed treatments [19].

A 3.8% SDF solution has the potential to be used as an antimicrobial root canal irrigant or interappointment dressing, especially in cases where the discolouration of dentin by metallic silver is not a major concern [20]. SDF has a high fluoride release capability and is a powerful anticariogenic agent. It can remineralise the tooth's surface and make it harder [21]. It has been promoted as a reasonable, effective, and safe caries-preventive agent and aligns with the World Health Organisation's Millennium goals [20].

A 3.8% SDF solution for irrigation was prepared by diluting the 38% SDF solution in a 1:10 ratio, as described in Hiraishi N et al.,'s laboratory study. This 3.8% SDF solution showed a 100% reduction in *E. faecalis* after 60 minutes of exposure, effectively removing the microorganisms present in the canal and surrounding dentin. However, the SDF solution caused discolouration of the root canal, and the number of precipitates on the pulpal dentin was correlated with the duration of SDF application. Sodium diamine fluoride can also be used as an inter-appointment dressing or root canal irrigator with antibacterial properties [20].

The SDF has an inhibitory effect on bacterial cell wall formation, division, and Deoxyribonucleic Acid (DNA) unwinding, significantly reducing the number of microbes in the root canal [19]. According to the findings of the study by Minavi B et al., 3.8% SDF, similar to 2% CHX, maintains substantivity within the dentinal tubules for a period of 3 weeks [22]. Mathew VB et al., also reported that SDF solution can effectively remove microbes from circumpulpal dentin when used as an endodontic irrigant [23]. As a result, 3.8% SDF was chosen as an irrigant in the current study, rather than 2% CHX. The findings of the investigation revealed a 66.8% decrease in CFU counts from baseline to post-instrumentation samples. The efficacy of antimicrobials, as indicated by the findings, was consistent with the investigation by Abrar E et al., [24]. According to a study by Hiraishi N et al., [20], 3.8% $Ag(NH_3)_2F$ was equally effective against microbes as 5.25% NaOCl. Similar statistically significant findings in the SDF group, matching those of the current investigation, were also discovered in the study by Maru V et al., [25].

When comparing the mean change from baseline to post-instrumentation for each sample between the study groups, there was no discernible difference between the 3.8% SDF and 2% CHX groups.

Results of an in-vitro investigation by Al-Madi EM et al., [8], comparing the antibacterial performance of 2% CHX and SDF as root canal irrigants against *E.faecalis*, revealed that SDF exhibited greater antibacterial efficacy than CHX. However, the results of the current study indicate no statistical difference when comparing the outcomes of each group.

### Limitation(s)

Limitations of the present study include the shorter application period of the irrigant (single-visit pulpectomy), which may have contributed to the equivalent efficacy of the two irrigants. Larger sample sizes and long-term follow-up investigations are necessary. Pulpectomies with multiple visits may yield better results in the 3.8% SDF category. No staining of the root canal was observed in the present study; however, the use of SDF might be restricted to posterior teeth only due to its unesthetic discolouration in anterior teeth. Additionally, further research is needed to determine the effectiveness of SDF against other bacteria in the root canal, as its efficacy has only been studied against *E.faecalis* so far.

## CONCLUSION(S)

Both the 3.8% SDF and 2% CHX groups demonstrated similar reductions in CFU/mL against *E.faecalis* in the current study. Based on these results, it is suggested that 3.8% SDF can be considered as a potential alternative root canal irrigant to 2% CHX. SDF is also known to exhibit substantivity and is more effective in completely eliminating *E.faecalis* from the root canal.

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