

Comparative Evaluation of the Efficacy of Chlorhexidine, Diode Laser, and Saline in Reducing the Microbial Count in Primary Teeth Root Canals: An *In Vivo* Study

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ABSTRACT

Context: Different irrigation materials in pediatric endodontics.

Aims: To evaluate the efficacy of chlorhexidine (CHX), diode laser, and saline in primary teeth root canals.

Settings and design: This manuscript is designed to know the effectiveness of the different root canal irrigants and laser irradiation technology in primary teeth root canals.

Materials and methods: A total of 60 primary teeth from 60 children were selected and divided into three groups of 20 each. Group I (CHX), group II (diode laser), and group III (saline). Pulp tissue was extirpated from the root canals of primary teeth, and samples were collected using sterile paper points. After cleaning and shaping, the root canals of the teeth in each group were disinfected using CHX and saline or irradiated with a diode laser. The samples were collected again and sent for microbiological examination.

Statistical analysis used: Statistical analyzes were performed using the Wilcoxon signed-rank test, Mann–Whitney *U* test, and Kruskal–Wallis one-way analysis of variance. All analyzes were conducted using the Statistical Package for the Social Sciences version 23.0.

Results: The colony-forming unit (CFU) of the pre-disinfection sample served as a baseline for comparisons throughout the study. The mean microbial counts of all the post-disinfected samples were reduced after disinfection. Intergroup comparisons showed significant results between groups I and III (CHX and saline) and groups II and III (diode laser and saline) ($p < 0.01$), whereas groups I and II (CHX and diode laser) showed nonsignificant results ($p < 0.092$).

Conclusion: Diode laser irradiation succeeded in reducing root canal infection when compared with 2% CHX. Diode laser irradiation can also be used for disinfecting the root canal in primary teeth.

Keywords: Chlorhexidine, Diode laser, Primary teeth root canal disinfection, Saline.

International Journal of Clinical Pediatric Dentistry (2023): 10.5005/jp-journals-10005-2596

INTRODUCTION

The main goal of pulp therapy in primary teeth is to maintain an intact dental arch, a healthy periodontium, whenever possible. The key determinants of the success of endodontic therapy depend on many elements like a precise diagnosis, in-depth canal cleansing, and, more importantly, an irrigation protocol.¹ Endodontic treatment for primary teeth is thought to be extremely difficult because of its unusual internal geometry and other characteristics like furcation connections and horizontal anastomoses.² The mechanical instrumentation approach has limited effectiveness in the elimination of bacterial colonization, which is present in the deeper layers and other irregularities in the root canal. The root canals of primary teeth are filled with a gelatinous mass of necrotic pulpal remnants and tissue fluids in the apical root portion. Hence, irrigation and instrumentation complement each other in complete debridement and disinfection of the root canals.³

As a result, irrigation and instrumentation work together to completely debride and disinfect the root. Hydrogen peroxide, CHX, sodium hypochlorite, and regular saline solutions are the most often utilized intracanal irrigation solutions.⁴

One of the most recent breakthroughs is cleaning root canals with lasers CO₂ erbium-doped yttrium-aluminium-garnet, neodymium-doped yttrium aluminium garnet, argon, and diode lasers, as well as photodynamic treatment are all options for root

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How to cite this article: Botu RK, Rachuri P, Martha S, *et al.* Comparative Evaluation of the Efficacy of Chlorhexidine, Diode Laser, and Saline in Reducing the Microbial Count in Primary Teeth Root Canals: An *In Vivo* Study. *Int J Clin Pediatr Dent* 2023;16(3):459–463.

Source of support: Nil

Conflict of interest: None

canal disinfection. It is advantageous to use a laser to treat the young patient for an oral and dental operation because it causes less anxiety in the child and is more well-liked by parents. Children become more cooperative when a doctor uses the laser for a surgical or pulpal operation, and it also improves the treatment result.⁵

The majority of the current literature concerns *in vitro* investigations on permanent teeth, with only a small amount including primary teeth. As a result, the current investigation was

conducted in primary teeth, which contain a far larger range of pathogens and had greater penetration into tubules than any *in vitro* system. The goal of this study was to see how effective CHX, diode lasers, and ordinary saline were effective at lowering the amount of bacteria in the root canals of primary teeth.

MATERIALS AND METHODS

Materials used in the study are shown in Table 1.

Inclusion Criteria

- Children who have previously experienced spontaneous pain.
- A tooth that is clinically nonvital and has a pus discharge.
- Teeth with continuous bleeding after coronal pulp tissue removal but good coronal structure.
- Deciduous teeth with at least two-thirds of root length.

Exclusion Criteria

- Teeth with necrotic pulps and radiolucency in the furcation area are advised for extraction.
- Primary teeth with root resorption of more than half of the total root length.
- Obliterated root canals.
- Within the past 2 months, the child had any systemic sickness, medically compromising circumstances, or had taken antibiotics.

A total of 60 primary teeth were selected from 60 children and placed into three groups of twenty each. Patients were instructed to pick a chit from a box with material names written on it for randomization. Prior to the commencement of the trial, the parents gave their informed consent.

In the current study, the following groups were formed:

- Group I: Irrigation was done with 2% CHX.
- Group II: Diode laser was used to irrigate the root canals.
- Group III: Irrigation was done with saline.

A single operator carried out the procedure. The rubber dam was placed after local anesthesia. In order to access the tooth's root canal and remove any cavities, the round bur with a high-speed handpiece was used under continuous irrigation with sterile distilled water. A sterile barbed broach was put into the tooth root

canal before any preinfection sample is collected, the extirpation of pulp done, then the paper point was inserted into the root canal to relax for 30 seconds after inserting into the root canal. The paper point was transferred immediately into the container containing 1 mL sodium thioglycolate medium, which serves as a transport medium for 30 seconds.⁶

Samples were collected from each of the three root canals for standardization. Using sterile K files, cleaning and shaping of the canals was done. Root canals in group I were irrigated with 2% CHX, group II root canals with diode laser, and group III with saline.

Group II's root canal irrigation procedure was done four times for 5 seconds using a flexible fiber tip of a diode laser with a 200 μm diameter (Denlase, 1.2 Watt) in the inbuilt mode of root canal sterilization. To avoid damaging the periapical region after the first irrigation, the fiber was placed into the root canal 1 mm short of the apex.

Under continual recessing circular motions, from the root canal's apical to coronal ends, the laser fiber is introduced. The laser cycles were separated by a 60 second interval of 5 seconds each. During laser irradiation, both the patient and the operator were wearing safety glasses.^{1,6}

The absorbent paper tip was inserted into the root canal and left to rest after completing cleaning and shaping the canals again. The paper tip was removed after 30 seconds and placed immediately into the sterile sample collecting container. After that, both preinfection and postinfection samples were sent to a microbiological lab.

The culturing was done at the Lenora Institute of Dental Sciences, Department of Microbiology. All of the obtained samples were vortexed before being incubated at 37°C for 2 hours. A micropipette was used to plate 10 μL of the material, which was subsequently placed on blood agar plates with a calibrated loop. The bacterial colonies on the labeled agar plates were counted after being incubated and cultured for 24–48 hours at 37°C. Both before and after the disinfection operation, CFUs were measured (Figs 1 to 3). The colony formation percentage decrease was measured, recorded, and tabulated.

RESULTS

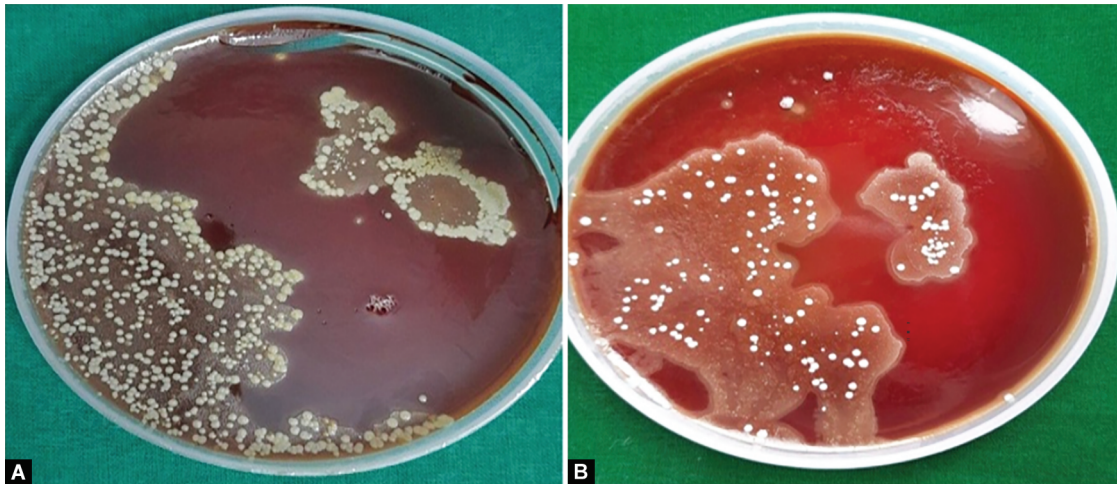
Table 2 shows the preinfection and postinfection descriptive statistics of the three groups, namely group I (CHX), group II (diode laser), and group III (saline) with equally distributed sample size, that is, ($n = 20$), which shows the mean values for all the three groups, among the preinfection samples for group I the mean value is 56.1, 14.27 is the standard deviation, the mean for group II 56.30 and the standard deviation is 18.70, and for group III the mean value is 60.30, and the standard deviation is 17.9.

Postinfection samples for group I, the mean value is 26.10, group II's mean value is 22.4 and its standard deviation is 19.81; group III's mean value is 55.80 and its standard deviation is 17.0; group III's mean value is 55.80 and in its standard deviation is 18.6, respectively, which shows the reduction of the microbial between the preinfection and postinfection samples.

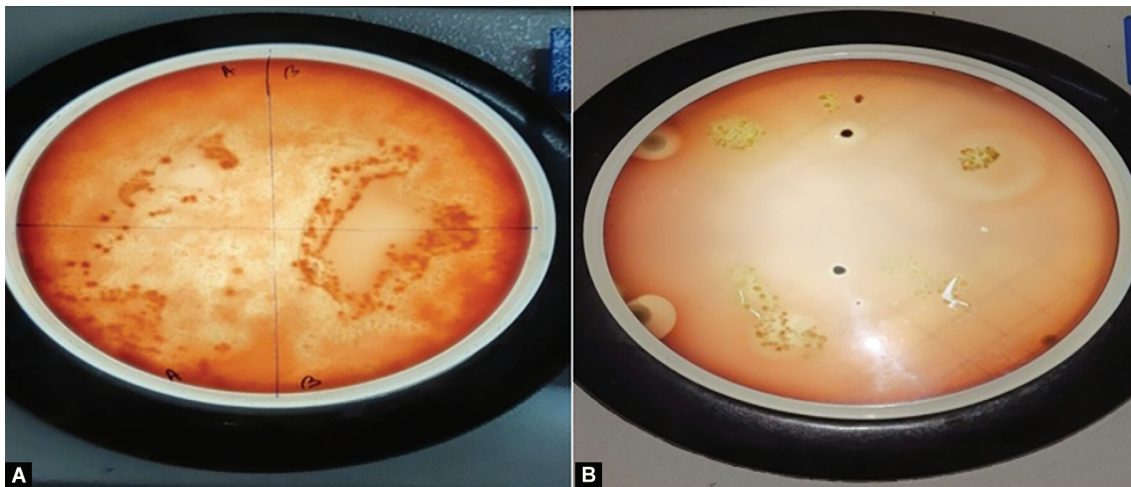
Table 3 shows the comparison using the Mann–Whitney *U* test, an intergroup comparison was made, and statistically significant differences were found between group I and group III (CHX and saline) and groups II and III (diode laser and saline) ($p < 0.01$) where the group I and group II (CHX and diode laser) showed the nonsignificant result ($p < 0.092$).

Table 1: The materials used in the study

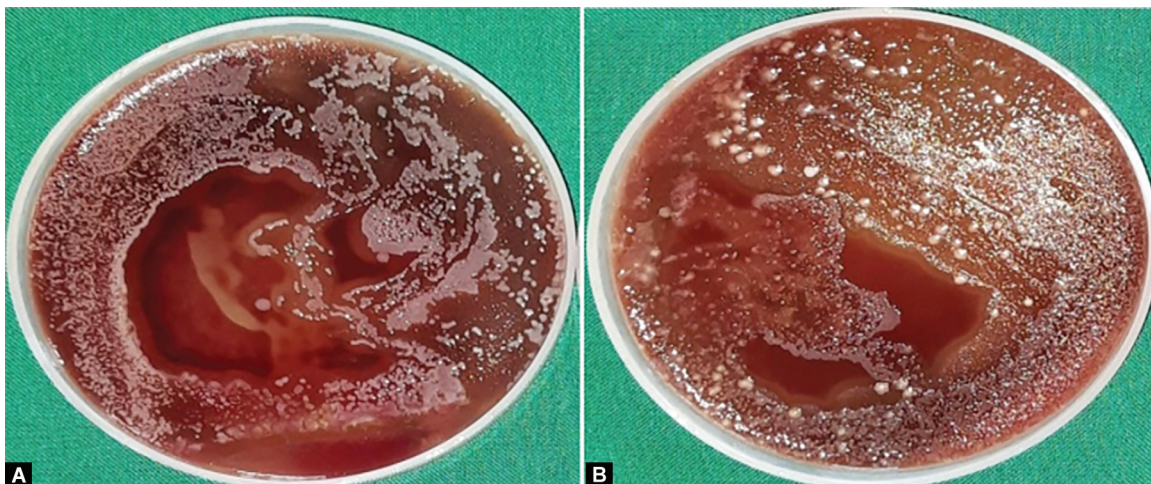
Petri dish
Eppendorf tubes
Blood agar
Thioglycolate
A 2% CHX gluconate solution
Normal saline
Diode laser
Biosafety cabinet
Anaerobic jar
Anaerobic gas pack
Inoculating loop
Armamentarium for pulp therapy
Local Anesthesia
Incubator
Digital colony counter



Figs 1A and B: Pre and postdisinfection with CHX



Figs 2A and B: Pre and postdisinfection with diode laser



Figs 3A and B: Pre and postdisinfection with saline

DISCUSSION

The establishment of an adequate level of disinfection in the root canals is important to the implementation of successful pulp therapy in primary teeth. Because a large amount of the diseased root canal remains undisturbed, disinfection with simply

mechanical equipment is not viable. Dentinal tubules and accessory canals contain necrotic pulpal tissue and dentin debris.¹

Shaping with instruments of the root canal system creates an amorphous layer of debris which includes dentinal chips, microorganisms, and their byproducts. The smear layer will occlude

Table 2: Comparison of the mean CFU of predisinfected and postdisinfected samples of three groups

	Groups	N	Mean CFU/mL	Standard deviation
Predisinfection	Group I CHX	20	56.1000	14.27512
	Group II diode laser	20	56.3000	18.70013
	Group III saline	20	60.3000	17.98274
	Total	60	45.4000	24.19637
Postdisinfection	Group I CHX	20	26.1000	17.06613
	Group II diode laser	20	22.4000	19.81068
	Group III saline	20	55.8000	18.64234
	Total	60	34.7667	23.65097

Table 3: Intergroup comparison of postdisinfection samples for group I (CHX), group II (diode laser), and group III (saline)

	Postdisinfection (group I CHX and group II diode laser)	Postdisinfection (group I CHX and group III saline)	Postdisinfection (group II diode laser and group III saline)
Mann–Whitney U	138.000	52.000	46.000
Wilcoxon W	348.000	262.000	256.000
Z	−1.685	−4.013	−4.176
p-value	0.092	0.000 HS	0.000 HS

and close the orifices of the dentinal tubules, thus preventing or delaying the action of irrigants and filling materials from immediate contact with the root surface dentin. Over a period of time, the loosely attached smear layer may disintegrate by the action of bacteria and their byproducts, leading to microleakage and reinfection. After the smear layer is removed, biocompatible irrigation solutions successfully eradicate microorganisms and their products, assisting the disinfection process for organic waste.¹

Chlorhexidine (CHX)'s efficacy as an antiplaque treatment has been demonstrated in a number of *in vivo* and *in vitro* experiments. The clinical reaction to CHX is dosage-dependent; frequency and concentration are critical in determining the clinical response. The recommended daily dose of CHX as a mouth rinse is 20 mg twice a day; however, equal levels of plaque inhibition can be reached with larger volumes of lower concentrations. Several investigations have shown that a lower concentration of CHX is beneficial. It was discovered that the bacteriostatic effect lasted for 12 hours.

The controlled release mechanism is credited with CHX's substantivity. The amount of CHX released is regulated and controlled by the presence of cyclodextrin. The more cyclodextrin there is, the more CHX is released gradually. Microfibrillated cellulose may also be used to build a controlled release mechanism from cellulosic substrates.

The mechanism of action of CHX is a matter of considerable debate. It has long been assumed that CHX is attached to the mucosal surfaces of the mouth and gradually releases over time. Jenkins et al. 1988, on the other hand, cast doubt on this mechanism, claiming that the main activity of CHX is due to the release of tooth-bound CHX rather than its oral retention or early bactericidal effects. One cation of CHX molecules may bind to the pellicle, leaving the other free to interact with bacteria colonizing the tooth surface. As a result, the process of bacterial suppression is carried out by CHX on the tooth surface.⁷

The use of diode lasers in root canal therapy has increased in recent years, owing to the lasers' antibacterial properties. The use of lasers in conservative dentistry and dental procedures has increased the likelihood of a satisfactory treatment outcome. The diseased pulp tissue and layers of root canal dentin may only be removed to a limited amount during traditional root canal therapy.

Chemical irritants are only effective in dentin layers right near to the canal wall, whereas root canal architecture restricts the amount of mechanical preparation. Bacteria can penetrate the peri luminal dentin up to 1000 μm whereas chemical disinfectants have a maximum penetration depth range of about 100–130 μm .^{8,9}

In the present study, 2% CHX solution, saline, and diode laser irradiation were done in the primary molar teeth root canals to know the efficacy of the irrigant, which is similar to the study done by Leonardo et al.¹⁰ where the authors concluded that because of its intracanal antibacterial action, 2% CHX gluconate can be utilized as an irrigating solution in root canals.

In another study conducted by Kaiwar et al.,¹¹ a 980 nm diode laser's ability to kill germs that have invaded dentin and so raise the success rate of endodontic therapy was concluded. All of the obtained samples were vortexed before being incubated at 37°C for 2 hours. A micropipette was used to plate 10 μL of the material, which was subsequently placed on blood agar plates with a calibrated loop. The labeled agar plates were incubated anaerobically in the anaerobic jar under anaerobic conditions using Gas Pack at 37°C for 24–48 hours.

According to a study by Pazelli et al.,¹² deciduous teeth's root canals with necrotic pulp and chronic periapical lesions displayed a high level of microorganisms and polymicrobial infection, with anaerobic bacteria predominating.

The microbial CFUs were counted using a digital colony counter for accurate results and to reduce the time. CFU/mL was measured before and after the use of the disinfection procedure. The percentage reduction in the microbial colony formation was measured, recorded, and tabulated.

The result of this study had shown the mean CFU of predisinfected samples of all three groups were high initially, and the mean CFU of all the postdisinfected samples was decreased, as shown in Table 1 and Figure 4. In the current study, the mean value of group I (CHX) in predisinfected sample was 56.1, group II (diode laser) was 56.3, and in group III (saline) was 60.3, the mean value of postdisinfected samples in group I (CHX) was 26.0, in group II (diode laser) 22.4 and group III (saline) 55.8, respectively.

The intergroup comparison of the present study showed statistically significant results ($p < 0.000$) shown in the

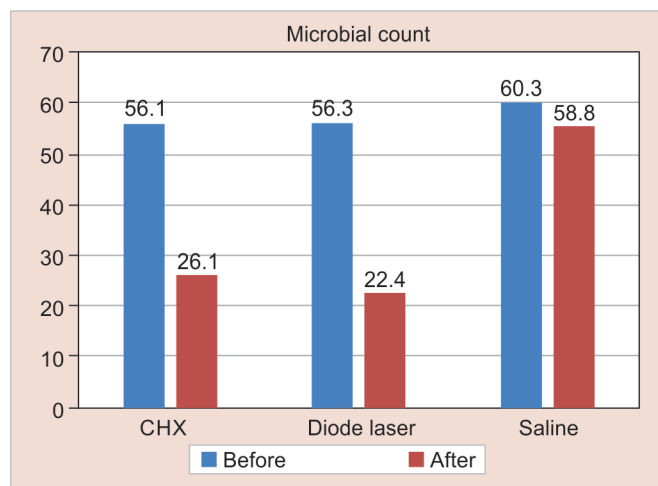


Fig. 4: Mean comparison of pre-disinfection and post-disinfection samples of three groups

post-disinfection group between the CHX and saline, diode laser, and saline. The results obtained in the present study can be correlated by the study done by Jain et al.,¹³ in which they found that on the intergroup comparison, a statistically significant difference was found between the saline and CHX group. Another study conducted by Louwakul and Prucksathamrongkul¹⁴ conducted a study at 6, 12, and 18 months, the NSS group's overall success rates were, respectively, 83, 93, and 97%. At 6, 12, and 18 months, the overall success rates in the CHX group were 100, 97, and 93%, respectively.

Similar statistically significant results were found when the 2% CHX and saline were used for the irrigation of the root canals in primary molars in the present study.

Ruiz-Esparza et al.¹⁵ observed a statistically significant difference in favor of 2% CHX gluconate when compared with saline. The study conducted by Walia et al.⁶ showed a statistically significant difference between the diode laser and saline groups. The mean reduction percentage in the diode laser group was 20.00 and in the saline group was 11.0, and the *p*-value was 0.004, respectively.

This can be correlated with the studies conducted by Sandini et al.,¹⁶ Walia et al.,⁶ and Ashofteh et al.¹⁷ In contrast, a study conducted by AA et al.¹⁸ results shows the highest change in the mean value percentage of the diode laser group when compared to the 2% CHX group. In comparison to the other groups, the diode laser disinfection was determined to be superior showing a total viable bacterial count.

Diode laser irradiation's stronger antibacterial effect could be ascribed to its deeper penetration when compared to a chemical disinfectant's insufficient ability to penetrate. According to this study's findings, disinfection with 2% CHX and diode laser in the primary tooth root canals dramatically reduced the microbial colony count, with the diode laser is more effective than 2% CHX. A diode laser is more efficient than 2% CHX. Irradiation with a diode laser can be an alternate method for disinfecting primary teeth root canals. Clinical research including root canal procedures on young children's teeth using a bigger sample size is also recommended.

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