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Antimicrobial efficacy of photodynamic therapy, diode laser, and sodium hypochlorite and their combinations on endodontic pathogens

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ABSTRACT

Background: Sterilization of the root canal system is of prime importance for a successful root canal therapy. Lasers and photodynamic therapy (PDT) have become the latest choice to eradicate microorganisms in the root canal.

Objective(s): To evaluate and compare the antimicrobial activity of diode laser, photodynamic therapy, and sodium hypochlorite along with their combinations on endodontic pathogens: *Enterococcus faecalis* and *Streptococcus mutans*.

Methods: A total of 120 uniradicular teeth were stored in 5.2% NaOCl solution to remove organic residues and the crowns were sectioned at the cemento-enamel junction (CEJ) to obtain root canal length of 15 mm. Canals were shaped using step-back technique up to #40 K-file and were autoclaved. The selected teeth (specimen) were randomly divided into two equal groups with sixty teeth being inoculated with *E. faecalis* (Group E) and remaining sixty teeth with *S. mutans* (Group S). Further, the groups were subdivided according to the disinfection technique used. Ten teeth from each subgroup were disinfected with a diode laser, photo activated disinfection (PAD), sodium hypochlorite, a combination of sodium hypochlorite and diode laser, a combination of sodium hypochlorite and photo activated disinfection respectively. Ten teeth in each group served as control without any disinfection. The treated specimens were transferred to test tubes containing 5 ml sterile Luria Bertani broth, incubated and the bacterial count, optical density in each root specimen was calculated and compared. The pairwise comparison of colonies across the subgroups was done by the Kruskal-Wallis test and within the subgroups was carried out using the Mann-Whitney U test. The statistical implication was tested at 5% and the study was performed using SPSS 18.0 ver. (SPSS Inc.).

Results: A significant reduction (98%) in the *E. faecalis* count was observed when the NaOCl was used in combination with the diode laser or PAD. PAD along with 3% NaOCl presents the advantage of utilizing a lower wavelength laser beam. Hence, PAD in combination with NaOCl can be an alternative and better option for root canal disinfection for both the endodontic pathogens, *E. faecalis* and *S. mutans*.

1. Introduction

Root canal infections consist of polymicrobial flora with roughly equal proportions of gram-positive and gram-negative bacteria [1,2]. Infected root canals have a variety of microorganisms like *Streptococci, Peptostreptococcus, Lactobacilli, Propionibacterium, Actinomyces, Eubacterium, Veillonella parvula, Bacteroides, Fusobacterium,* etc [3]. For a successful root canal therapy, sterilization of the root canal system is of prime importance.

The accomplishment of any endodontic treatment strongly relies on the chemo-mechanical removal of microorganisms and pulp debris using instruments in biomechanical preparations and irrigating solutions [4]. Irrigating solutions used during endodontic handling act through undeviating contact with the bacteria targeted. However, irrigants have inadequate penetration depth. It is highly desirable that chemical substances selected as endodontic irrigants have antimicrobial and organic tissue dissolution properties besides serving in the debridement of the root canal system and not being toxic to the tissues [5].

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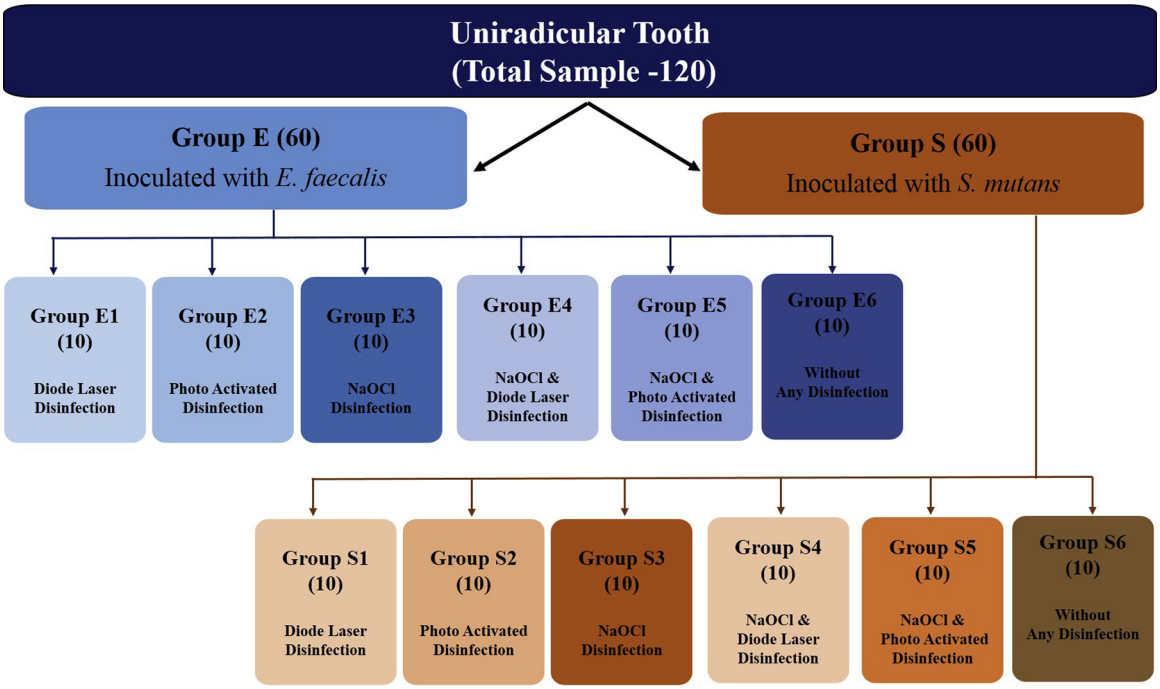

Fig. 1. Distribution of the samples.

Ideal root canal irrigant solution does not exist because no endodontic irrigant can fulfill all the ideal requirements including host tissue response, tissue solvent property, antimicrobial effect and cost [6]. Sodium hypochlorite (NaOCl) has been used as an endodontic irrigating solution by the greater part of professionals.

Lasers have become the latest choice to eradicate microorganisms in the root canals, especially in the lateral dentinal tubules. This has been achieved by the development of a fiber delivery system. It has been proven that a release of laser light directly in the root canal has a bactericidal effect [7].

Photodynamic therapy (PDT) which consists of three components (oxygen, photosensitizer, and light) is a treatment that utilizes light to activate a photosensitizing agent (photosensitizer) in the presence of oxygen. The exposure of the photosensitizer to light results in the formation of oxygen species, such as singlet oxygen and free radicals, causing localized photodamage and cell death [8].

NaOCl, Diode laser and, PDT when used individually have few limitations or drawbacks. A combination of these disinfectants may be beneficial. However, studies on the combinations of disinfectants are scanty.

Hence the present study was designed to evaluate and compare the efficacy of NaOCl, Diode laser and PDT along with their combinations on the most resistant and commonly found microorganism in diseased dental pulp i.e. *Enterococcus faecalis* and *Streptococcus mutans* by an in-vitro method. The study was permitted by the ethical clearance board where the study was conducted.

2. Materials and methods

2.1. Source of data

One hundred and twenty uniradicular permanent teeth extracted because of periodontal disease or for orthodontic purposes were selected for the study. Uniradicular teeth with completely developed root, teeth with a single root canal were included for the study. Multirooted teeth, teeth with root caries and teeth with a history of endodontic treatment were excluded from the study.

2.2. Sample preparation

One hundred and twenty extracted uniradicular teeth were stored in 5.2% NaOCl solution for 30 min to eradicate organic residues and were left in saline solution until the procedure began. The crowns were sectioned at the cemento-enamel junction (CEJ) using a high-speed diamond disk to get hold of root canal length of 15 mm. K-file #10 (Dentsply-Maillefer) was introduced into each canal until it appeared at the apical foramen. The working length was recognized by subtracting 0.5 mm from this length. The canals were shaped using the step-back technique up to #40 K-file (Dentsply Maillefer). Five ml of 3% sodium hypochlorite irrigating solution was used after each instrumentation. The teeth were then air-dried all night at room temperature and apical foramen was sealed externally and waterproofed with coats of clear nail varnish. The teeth were sterilized in the autoclave at 121 °C for 15 min under 15 lbs pressure.

2.3. Distribution of the samples

E. faecalis (MTCC 439) and *S. mutans* (MTCC 497) were obtained from IMTech, Chandigarh, and routinely maintained on nutrient agar slants at 4 °C. An overnight liquid culture of the organisms in Luria Bertani (LB) broth was used in all the experiments. Sterile LB broth was inoculated with the test organisms and incubated at 37 °C overnight. The cell density was adjusted to approximately10^9 cells/ml which corresponded to an absorbance of 1.0 at OD_{600} for all experimental purposes.

The teeth selected for the study were randomly divided into two equal groups with sixty root canals inoculated with *E. faecalis* (Group E) and the remaining sixty root canals with *S. mutans* (Group S). Further, ten teeth each from both the groups were disinfected as follows (Fig. 1):

Group E1 and Group S1: Disinfection of the root canals with a diode laser.

Group E2 and Group S2: Disinfection of the root canals with PAD.

Group E3 and Group S3: Disinfection of the root canal with sodium hypochlorite.

Group E4 and Group S4: Disinfection of the root canal with sodium hypochlorite and diode laser.

Group E5 and Group S5: Disinfection of the root canal with sodium hypochlorite and PAD.

Group E6and Group S6: Control group undergoing no disinfection.

2.4. Procedure of disinfection

2.4.1. Diode laser disinfection

Specimens of Group E1 and Group S1 were treated with a diode laser (Doctor smile) with energy set at 1.5 W, central wavelength of 980 nm irradiation followed oscillatory technique developed by Gutknecht et al [9]. The optical fiber was introduced 1 mm short of the apex and was recessed in helicoidal movements at a speed of around 2 mm/s for 5 s, repeated 4 times at intervals of 10 s, between each one. This rest period between irradiation avoided temperature change. Post laser treatment, the teeth were positioned in vials, which contained 2 ml of the nutrient broth.

2.4.2. Photo-Activated disinfection (PAD)

Specimens of Group E2 and Group S2 were treated with PAD. Methylene blue was dissolved to the concentration of 25 μg/ml (67μM) before the use. The diode laser was the source of irradiation with an output power of 2 W and a central wavelength of 660 nm. Irrigation needle of 30 gauge was used to fill the root canal with a methylene blue solution. The whole sample was then fully enclosed by methylene blue for five minutes and was then aspirated from the root specimens. Light activation was done using a flexible optical fiber with cylindrical diffusers (*Cuda Technologies Inc., Jacksonville, FL*) that were connected to the diode laser in helicoidal movements at a speed of approximately 2 mm/s for five seconds continuous mode with random polarization along with 10 J radiant energy. It was repeated 4 times with an interval of 10 s, between each one. The optical fiber was placed 1 mm short of the apex and the rest period between irradiation was given to avoid temperature changes. The optical fiber with a diameter of *500* μm used was able to allocate light uniformly at 360 degrees to an extent of 15 mm. After disinfection, teeth were placed in vials containing 2 ml of nutrient broth.

2.4.3. Sodium hypochlorite (NaOCl) disinfection

Specimens of Group E3 and Group S3 were treated with 3% NaOCl for disinfection. Initially, 0.6 ml of NaOCl was used for irrigation for 20 s, followed by hand agitation (extending up to the working length) with 20 No. K file (Dentsply Maillefer) for 100 s. This was continued five times to give a total of 3.0 ml of irrigant distributed over 10 min.. After disinfection, teeth were placed in vials containing 2 ml of nutrient broth.

2.4.4. NaOCl and diode laser disinfection

Specimens of Group E4 and Group S4 were initially disinfected with NaOCl, followed by Diode laser disinfection. The tooth specimens were then positioned in vials containing 2 ml of nutrient broth.

2.4.5. NaOCl and PAD

Specimens of Group E5 and Group S5 were initially treated with NaOCl disinfected followed by the photo activated disinfection. The tooth specimens were then positioned in vials containing 2 ml of nutrient broth.

2.4.6. Control group without any disinfection

Specimens of Group E6 and Group S6 did not undergo any disinfecting procedure and were taken as a control group for the research.

2.5. Bacteriological analysis

The treated specimens were transferred to test tubes containing 5 ml sterile LB broth and incubated at 37 °C for 24 h.

Aliquots were withdrawn from each tube, serially diluted and 100 μl samples used for estimating CFU/ml (colony-forming unit per milliliter) by standard pour plate method. Also, the OD at 600 nm was measured for each tube. The bacterial count and OD_{600} of the treated specimens were compared with that of the respective control (untreated) group (Group E6 and Group S6). Survival fractions in each root specimen were calculated by counting the colonies on the experimental plates and dividing by the number of colonies from controls.

The data on counts of endodontic pathogens i.e. *E. faecalis* and *S. mutans* were obtained for ten samples from each of the study groups. The mean and median values of counts were obtained. The comparison of data across the subgroups was performed using the Kruskal-Wallis test. The pairwise comparison of counts within the subgroups was carried out using the Mann-Whitney U test. The statistical implication was tested at 5% and the study was performed using SPSS 18.0 ver. (SPSS Inc.).

The log reductions in the bacterial count were calculated as Log Reduction = $\log_{10}$ (A/ B)

Where 'A' denotes the number of viable microorganisms before treatment and 'B' denotes the number of viable microorganisms after treatment.

3. Results

3.1. E. faecalis count across the study groups

In the current study the mean ± standard deviation of CFU's of *E. faecalis* for Group E1, Group E2, Group E3, Group E4, Group E5 and Group E6 were 0.223 ± 0.053, 0.250 ± 0.027, 0.247 ± 0.031, 0.014 ± 0.005, 0.019 ± 0.006 and 0.921 ± 0.079 respectively and was found to be highly significant ($p < 0.0001$) across the study groups (Fig. 2).

3.2. S. mutans count across the study groups

The mean CFU's of *S. mutans* for Group S1, Group S2, Group S3, Group S4, Group S5, and Group S6 were 0.125 ± 0.03, 0.144 ± 0.023, 0.127 ± 0.025, 0.056 ± 0.02, 0.050 ± 0.018 and 1.521 ± 0.661 respectively and was also found to be highly significant ($p < 0.0001$) across the study groups (Fig. 3).

3.3. Comparison of E. faecalis count (CFU's) within the study groups

There was a highly significant difference when Group E1 was compared with Group E4, Group E5, and Group E6. However, no statistical difference was seen when Group E1 was compared with Group E2 and Group E3 (Table 1).

A significant difference was not observed when Group E2 was compared with Group E3. However, Group E2showed a highly significant difference when compared with Group E4, Group E5, and Group E6. Group E3 showed a highly significant difference when compared to Group E4, Group E5, and Group E6.

Group E4 and Group E5 showed a highly significant difference when they were compared to Group E6. There was no significant difference when Group E4 was compared with Group E5.

3.4. Comparison of S. mutans count (CFU's) within the study groups

There was a highly significant difference when Group S1was compared with Group S4, Group S5, and Group S6. However, no statistical difference was seen when Group S1 was compared with Group S2 and Group S3. Statistical difference was not seen when GroupS2 was compared with Group S3. However, GroupS2 showed a highly significant difference when compared with Group S4, Group S5, and Group S6 (Table 2).

Group S3 showed a highly significant difference when compared to Group S4, Group S5, and Group S6. Group S4 and Group S5 showed a

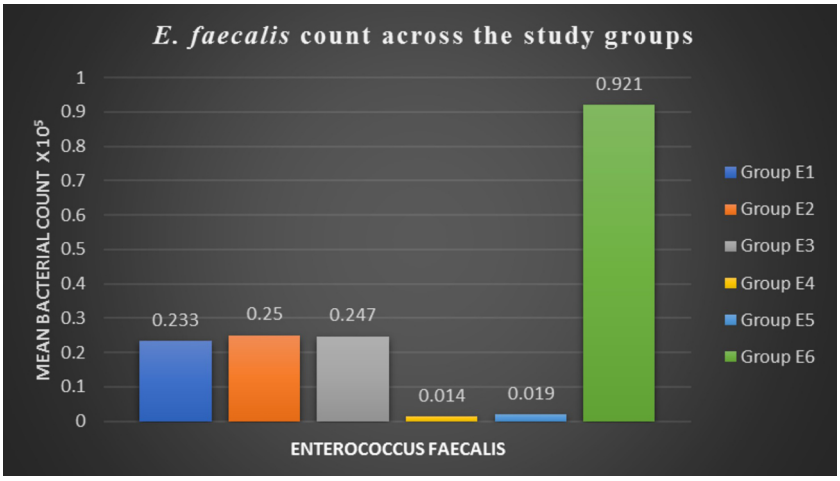

Fig. 2. E. faecalis count across the study groups.

highly significant difference when they were compared to Group S4. However, there was no significant difference when Group S4 was compared with Group S5 (Table 2).

3.5. Reduction in E. faecalis count

The mean percentage reduction in *E. faecalis* count post-treatment for Group E1, was 76% whereas Group E2 and Group E3 showed 73%. 98% (1.69 logarithm) reduction in the *E. faecalis* count was observed when the NaOCl was used in combination with diode laser or PAD suggesting that the combination therapies have a better antibacterial efficacy against *E. faecalis* than the therapies used individually (Table 3).

3.6. Reduction in S. mutans count

The mean percentage reduction in *S. mutans* count post-treatment for Group S1, Group S2, Group S3 were 92%, 91% and 92% respectively while 96% (1.40 logarithm) and 97% (1.53 logarithm) reduction in *S. mutans* count was observed when the combination of therapies was used suggesting that the combination therapies have a better antibacterial efficacy against *S. mutans* than the therapies used individually (Table 4).

4. Discussion

Microorganisms are competent in invading the peri-luminal dentin up to a depth of 1100 µm, whereas the chemical irrigants penetrate no more than 130 µm into the dentin [10,11]. Microorganisms in a failed root canal cases are diverse from those present in an infected root canal before the endodontic treatment [12].

Enterococcus faecalis, a gram-positive facultative anaerobic coccus present in oral flora is always identified in persistent root canal infections and is also associated with the failure of endodontic treatment [13]. *E. faecalis* survives as a lonely organism in the root canals without the support of other bacteria and is resistant to high temperatures [14–16].

Streptococcus mutans are facultatively anaerobic, gram-positive coccus bacterium frequently established in the oral cavity which are the main contributors for caries [17]. Pazelli et al. reported the presence of streptococci in infected root canals with periapical infections [18]. Hence, *E. faecalis* and *S. mutans* were selected as the pathogens in the present study.

The use of lasers in endodontic therapy has been widely studied and laser treatments have been proven to have many advantages over conventional methods. The high-power diode laser decreases dentine permeability, even though it does not provoke the dentine melting unlike neodymium laser [19]. The diode laser device is composed of two layers of semiconductor material interlaced with a non-conductive layer. It's light spectrum of diode laser allows for better absorption by

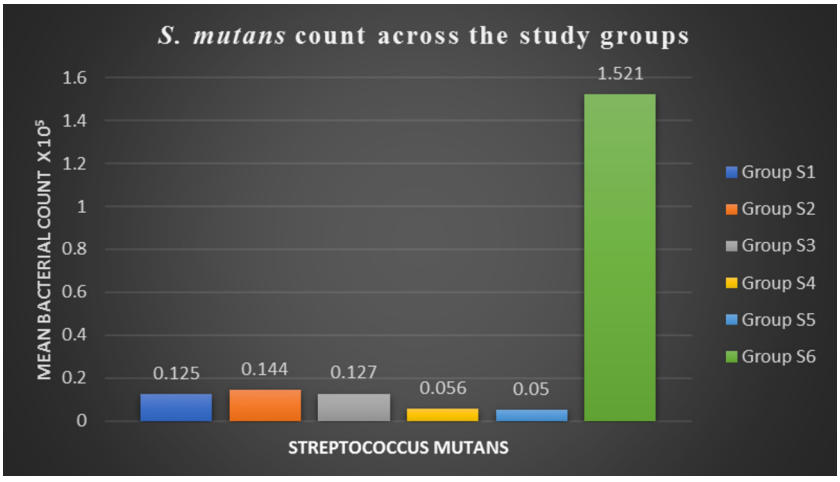

Fig. 3. S. mutans count across the study groups.

Table 1
Comparison of *E. faecalis count* (CFU's/ml) within the study groups.

Groups		No. of samples	*E. faecalis* (CFU/ml $\times 10^5$)		*P*-value
			Mean ± (SD)	Median	
Group E1		10	0.223 ± (0.053)	0.245	
vs	**Group E2**	10	0.250 ± (0.027)	0.255	0.3230
	Group E3	10	0.247 ± (0.031)	0.255	0.3624
	Group E4	10	0.014 ± (0.005)	0.014	< **0.001***
	Group E5	10	0.019 ± (0.006)	0.021	< **0.001***
	Group E6	10	0.921 ± (0.079)	0.938	< **0.001***
Group E2		10	0.250 ± (0.027)	0.255	
vs	**Group E3**	10	0.247 ± (0.031)	0.255	0.879
	Group E4	10	0.014 ± (0.005)	0.014	< **0.001***
	Group E5	10	0.019 ± (0.006)	0.021	< **0.001***
	Group E6	10	0.921 ± (0.079)	0.938	< **0.001***
Group E3		10	0.247 ± (0.031)	0.255	
vs	**Group E4**	10	0.014 ± (0.005)	0.014	< **0.001***
	Group E5	10	0.019 ± (0.006)	0.021	< **0.001***
	Group E6	10	0.921 ± (0.079)	0.938	< **0.001***
Group E4		10	0.014 ± (0.005)	0.014	
vs	**Group E5**	10	0.019 ± (0.006)	0.021	0.0689
	Group E6	10	0.921 ± (0.079)	0.938	< **0.001***
Group E5		10	0.019 ± (0.006)	0.021	
vs	**Group E6**	10	0.921 ± (0.079)	0.938	< **0.001***

* Highly Significant, CFU – Colony Forming Unit.

water in the dental tissues when compared with Nd: YAG laser. This attributes to the greater penetration capacity of laser light into the dentin, further into the dentinal tubules to target the microorganisms and hence used for the present research.

Moritz et al. recommended that the diode laser could be measured equal to the Nd: YAG laser in endodontic treatment [20]. They conducted an in-vitro study with an 810 nm wavelength diode laser (4 W power, every time for 5 s) to reduce intra canal microorganisms and concluded that this laser is effective for sealing dentinal tubules and eliminating *Escherichia coli* and *Enterococcus faecalis* [20]. The diode laser (Group E1 and S1)) used in the present research is of 910 nm wavelength (2 W power) introduced 2 mm short of the apex and was recessed with a speed of 2 mm/s for 5 s in helicoidal movements. It was repeated with an interval of 10 s, between each one four times. The rest period between irradiation avoided temperature changes.

Diode laser with 810 nm wavelength, 3 W power on continuous mode (CW) for 30seconds was used to eliminate *E. faecalis* and a moderate 74% reduction in the number of bacteria for a dentinal thickness of 500 microns was observed [9]. In the present study, a 76% reduction in the *E. faecalis* and 92% reduction in *S. mutans* were observed which is in accordance with the earlier studies which reported that diode lasers are not very effective in complete eradication of E. faecalis [9,21]. Complete eradication of *E. faecalis* was accomplished with high power irradiation but produced a high temperature on the root surface [20].

Laser's power density is required to determine whether a beam could damage an optic or sensor or for various other applications. It is nothing but the power per unit area which is usually expressed in kW/cm^2. Power density can easily be obtained by calculating the area of a beam using a radius in cm and dividing the beam's power by that area.

Table 2
Comparison of *S. mutans* count (CFU's/ml) within the study groups.

Groups		No. of samples	*S. mutans* (CFU/ml $\times 10^5$)		*P*-value
			Mean (SD)	Median	
Group S1		10	0.125 ± (0.030)	0.125	
vs	**Group S2**	10	0.144 ± (0.023)	0.150	0.1707
	Group S3	10	0.127 ± (0.025)	0.125	0.9092
	Group S4	10	0.056 ± (0.020)	0.055	< **0.001***
	Group S5	10	0.050 ± (0.018)	0.051	< **0.001***
	Group S6	10	1.521 ± (0.661)	1.540	< **0.001***
Group S2		10	0.144 ± (0.023)	0.150	
vs	**Group S3**	10	0.127 ± (0.025)	0.125	0.1583
	Group S4	10	0.056 ± (0.020)	0.055	< **0.001***
	Group S5	10	0.050 ± (0.018)	0.051	< **0.001***
	Group S6	10	1.521 ± (0.661)	1.540	< **0.001***
Group S3		10	0.127 ± (0.025)	0.125	
vs	**Group S4**	10	0.056 ± (0.020)	0.055	< **0.001***
	Group S5	10	0.050 ± (0.018)	0.051	< **0.001***
	Group S6	10	1.521 ± (0.661)	1.540	< **0.001***
Group S4		10	0.056 ± (0.020)	0.055	
vs	**Group S5**	10	0.050 ± (0.018)	0.051	0.5197
	Group S6	10	1.521 ± (0.661)	1.540	< **0.001***
Group S5		10	0.050 ± (0.018)	0.051	
vs	**Group S6**	10	1.521 ± (0.661)	1.540	< **0.001***

* Highly Significant, CFU – Colony Forming Unit.

Table 3
Reduction in *E. faecalis* count.

Groups	No. of samples	*E. faecalis* counts (CFU/ml × 10^5)		Logarithmic Reduction
		Mean ± (SD)	Decrease in Count (%)	
Group E1	10	0.223 ± (0.053)	76	0.62
Group E2	10	0.250 ± (0.027)	73	0.57
Group E3	10	0.247 ± (0.031)	73	0.57
Group E4	10	0.014 ± (0.005)	98	1.69
Group E5	10	0.019 ± (0.006)	98	1.69
Group E6	**10**	0.921 ± (0.079)	–	–

CFU – Colony Forming Unit.

In the present study, it ranged from 0.75 to 1.02 kW/cm^2. Methylene blue of phenothiazine family with a well-recognized photosensitizing property has been extensively used in photodynamic therapy [22]. In Group E2 and Group S2 of the present study, methylene blue was dissolved to the concentration of 25 µg/ml (67µM) before the use. The irradiation source used in the present study was a diode laser with an output power of 2 W and a central wavelength of 660 nm. 73% and 91% reduction in *E. faecalis* and *S. mutans* respectively were noted.

The antibacterial result of a laser is based on the thermal properties of laser-tissue interaction [23]. The diode laser has proved its efficacy for disinfection, providing a way into previously inaccessible parts of the tubular network, due to the better penetrating capacity than the irrigation solutions. The huge advantage of using PDT is that the organism cannot create resistance against it [24].

Soukos et al. reported that *E. faecalis* when exposed to 25 g/ml methylene blue followed by subsequent illumination with light resulted in a 97% reduction in viable cells. Specimens treated with only methylene blue showed 83.2% ($p = 0.009$) reduction, whereas with light alone it showed 56.6% ($p = 0.03$) reduction [25]. Similar findings were reported by Bumb et al. with a 96.7% reduction of *E. coli* by using methylene blue in PDT along with the diode laser (910 nm wavelengths with power settings of 1 W, at a pulsed mode) [26]. In the present study, failure to eradicate microorganisms especially E. coli may be attributed to the laser of low wavelength (660 nm) used for irradiation in Group E2. However, various types of light and drug/dye parameters must be further explored to explain the appropriate dosimetry to completely eradicate root canal microorganisms.

Sodium hypochlorite (NaOCl) is the most commonly used root canal disinfectant solution. NaOCl ionizes in water into Na^+ and the hypochlorite ion, OCl^-, establishing equilibrium with hypochlorous acid (HOCl). Sodium hypochlorite, the gold standard irrigant in endodontics, is used in 0.5%–6% concentrations commonly. It has strong antibacterial and tissue dissolving effects but has toxic effects on periapical tissue and thus must be used with caution. It is believed that it degrades micromechanical characteristics of dentin but has no effect on the inorganic part of the smear layer [27,28].

Observations of the present study showed that 3% NaOCl (Group E3 and S3) was used within the working length over 20 s, followed by hand agitation with a size 25 no. K file (Dentsply Maillefer), lengthening to the working length for 100 s. This was repeated five times to give a total of 3.0 ml of irrigant delivered over 10 min.. The total reduction in *E. faecalis* and *S. mutans* was observed to be 73% and 92% respectively. Thomas et al. compared the antimicrobial property of diode laser, sodium hypochlorite and Triphala in primary root canals and concluded that laser almost completely eradicated *E. faecalis*. Triphala showed better antimicrobial properties than sodium hypochlorite but inferior to lasers [29].

NaOCl, which is taken as the Gold Standard in endodontics showed much better results and efficacy in reducing the bacterial count when used in combination with diode laser or PAD than when used unaided in the present study.

In studies where diode laser was used in combination with canal irrigating solutions like oxygenated water and sodium hypochlorite, better results were obtained [9,30]. In the present study when the diode laser was used in combination with the NaOCl we found 98% and 96% reduction in the *E. faecalis* and *S. mutans* suggesting that the application of the diode laser might be an adjunct to conventional endodontic treatment when used in combination with a NaOCl solution.

Similarly, when photo-activated disinfection was used in combination with NaOCl there was a reduction by 98% and 97% of *E. faecalis* and *S. mutans*. A study conducted by Vezzaniet al with Er: YAG laser at different frequencies found a microbial reduction of 85.33% for the group irradiated with Er:YAG laser at 100 mJ/7 Hz, 74.58% at 100 mJ/10 Hz, and 89.50% at 100 mJ/16 Hz and when treated in combination with 1.0% and 2.5% NaOCl solution, 83.15% and 84.46% values of microbial reduction were obtained respectively [31].

Castelo-Baz et al. conducted a study to evaluate the bactericidal efficacy of a 940 nm diode laser alone and in combination with 5% NaOCl solution against *E. Faecalis*. They concluded that the combination of diode laser (940 nm) and NaOCl had a synergistic effect, intensifying the bactericidal action [32].

Similarly, Vaid et al. compared the combined effect of PAD and 2.5% s NaOCl solution with saline and NaOCl irrigation alone on *E. faecalis*. They concluded that PAD with 2.5% NaOCl was the most effective in disinfecting root canals containing mature *E. faecalis* biofilms [33]. However, a diode laser of 980 nm wavelength with an output power of 1.5 W was used. One of the interesting findings in the present study was that 98% reduction of *E. faecalis* was seen despite using PAD with a lower wavelength (660 nm) diode laser along with a 3% NaOCl

Table 4
Reduction in *S. mutans* count.

Groups	No. of samples	*S. mutans* counts (CFU/ml × 10^5)		Logarithmic Reduction
		Mean ± (SD)	Decrease in Count (%)	
Group S1	10	0.125 ± (0.030)	92	1.09
Group S2	10	0.144 ± (0.023)	91	1.04
Group S3	10	0.127 ± (0.025)	92	1.09
Group S4	10	0.056 ± (0.020)	96	1.40
Group S5	10	0.050 ± (0.018)	97	1.53
Group S6	10	1.521 ± (0.661)	–	–

CFU – Colony Forming Unit.

irrigating solution.

The possible reason for the antibacterial activity of NaOCl could be attributed to its high pH (hydroxyl ion ions action). NaOCl causes biosynthetic alterations in cellular metabolism and phospholipid destruction. Cellular metabolism is interfered with by the formation of chloramines, with oxidative action with irreversible enzymatic inactivation in bacteria, fatty acid and lipid degradation [30].

Additive bactericidal effect is seen when combined therapy ie. either laser and NaOCl or PAD and laser (660 nm) can be attributed to the increased depth of penetration of laser into dentinal tubules [33].

Photodynamic therapy (PDT) works on two mechanisms. The type I mechanism involves the transmission of the energy of photoactive chemical compounds, known as photosensitizers (PSs) to a substrate or biomolecule, and, from this intermediate, the energy is forwarded to oxygen, giving rise to radical oxygen species (ROS). The type II mechanism involves the injection of the PS into tissues and then irradiated at a certain wavelength to reach an excited energy level. The absorbed energy can then be transferred directly to neighboring molecules, such as O2, giving rise to singlet oxygen, which in turn gives rise to ROS. In both cases, ROS induce cell death by apoptosis or necrosis, thus making PDT interesting for the treatment of several diseases [34–37]. The success in eradication of microorganisms in the present study may be attributed to the above mechanism of action of PAD.

Photoactivated disinfection in combination with sodium hypochlorite offers many advantages like quick and rapid penetration of the drug into the root canal killing the bacteria in a short time; complete penetration of photosensitizers into the biofilms and dentinal tubules; limited penetration and cytotoxicity of photosensitizers and light into the adjoining bone and periodontal ligament; and absence of thermal side effects in the tissues close to the roots [8,9,22]. PAD with low wavelength diode lasers along with NaOCl (Group E5 and Group S5) proved to be equally effective on *E. faecalis* and *S. mutans* when compared to high wavelength lasers used along with NaOCl (Group E4 and Group S4).

The rationale for performing an in vitro antibacterial efficacy is to offer the clinician valuable information regarding the antibacterial efficacy of diode laser, photoactivated disinfection and sodium hypochlorite along with their combinations. Consequently, to determine the true antibacterial effectiveness, in vivo testing to evaluate the role of various components in these irrigants are very essential.

5. Conclusions

From this research, we can conclude that the combination of an irrigating solution with diode laser or photo activated disinfection (PAD) will provide a better efficacy in reducing the pathogenic count. PAD presents with an advantage of utilizing the laser beam of lower wavelength thereby masking the disadvantage of laser and hence can be utilized safely for the periapical tissues without causing much injury to the peripheral tissues and bones.

The results of the present study suggest that PAD along with 3% NaOCl can be an alternative and better option for root canal disinfection.

Authors contributions

Author 1: Acquisition of data or analysis and interpretation of data; and Drafting the article and Final approval of the version to be published.

Author 2: Concept and design of study, data analysis and interpretation of data; Drafting the article or revising it critically for important intellectual content; and Final approval of the version to be published.

Author 3: Acquisition of data, revising it critically for important intellectual content; and Final approval of the version to be published.

Author 4: Acquisition of data, revising it critically for important intellectual content; and Final approval of the version to be published

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Declaration of Competing Interest

Nil.

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