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ANTIBACTERIAL EFFECT OF DIODE LASER 635NM, OZONATED WATER, AND *NIGELLA SATIVA* OIL AS ROOT CANAL IRRIGANTS AGAINST *ENTEROCOCCUS FAECALIS* IN PRIMARY TEETH

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Abstract

Background: Root canal irrigants play an important role in pediatric endodontics due to bizarre internal configurations such as horizontal anastomoses and internal connections. Sodium hypochlorite is the most effective irrigant solution, but its potential toxicity issues necessitate the search for new alternatives. **Aim**: to compare the antibacterial effectiveness of sodium hypochlorite, 635 nm diode laser, ozonated water, and *Nigella sativa* as root canal irrigants against *Enterococcus faecalis* in deciduous teeth. **Design**: A total of 450 extracted primary anterior teeth were used. For 24 hours, specimens were infected with *E. faecalis*, then allocated into seven test groups randomly, including 2.5% Sodium hypochlorite (NaOCl), diode laser 635 nm, ozonated water, *Nigella sativa* oil, diode laser-NaOCl, diode laser-ozonated water, diode laser-*Nigella sativa* oil, and two control groups. After disinfection, all specimens were agitated with sterile saline solution, and paper points #30 were inserted into the canals to inoculate the bacteria on agar plates. Subsequently, methylene blue was inserted into the canals and activated according to the groups. The histological ground sections for all teeth were done and evaluated under the light microscope. **Results**: The highest percentage of bacterial reduction was 97.46% for the diode-*Nigella sativa* group, without a statistically significant difference from other groups. **Conclusions:** When combined, the 635 nm diode laser and *Nigella sativa* oil can eliminate *E. faecalis* from primary root canals.

Keywords: *Enterococcus faecalis*, 635 nm diode laser, Sodium hypochlorite, Ozonated water, *Nigella sativa, dentine penetration*.

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1. INTRODUCTION

The primary goal of pulpectomy in the primary dentition is to preserve every primary tooth as an entirely functional element of the dental arch, allowing for appropriate mastication, speech, swallowing, and maintenance of the space needed for permanent teeth eruption, as well as the avoidance of psychological impact associated with loss of teeth [1]. The proper use of good instrumentation, irrigation, and root canal obturation are crucial factors of successful root canal therapy [2]. The primary teeth pulpectomy is a tricky issue especially due to anatomical variants like curved and convoluted root canals, their closeness to developing tooth buds, and a decrease in awareness of the impacts of root canal irrigants [3].

Root canal irrigants play a significant role in pediatric endodontics due to the peculiar internal configuration such as internal connections and horizontal anastomoses. It has many roles that differ greatly depending on the irrigant used; it minimizes friction between the instrument and the dentine, cools the file and tooth, particularly with using ultrasonic energy, enhances the cutting potency of the files, dissolves tissue, and has a cleaning and antiseptic effect [4]. Furthermore, irrigation is the only means of reaching regions of the root canal wall that is unattainable by mechanical instrumentation [5].

The microorganisms found in the root canals of permanent teeth are similar to those found in primary root canals. The most common bacterial species found in infected primary tooth root canals were *Enterococcus faecalis* (*E. faecalis*), *Porphyromonas gingivalis, and Treponemadenticola* [6] . Four to forty percent of all primary endodontic infections are caused by *E. faecalis*, one of the most frequent bacteria in recurrent root canal infections. This species is the most challenging to treat and could be the reason for root canal failure. *E. faecalis* features that cause its resistance to chemo-mechanical irrigation, including adherence to collagen fibres, deep penetration into dentinal tubules, high pH tolerance, sustenance in the apparent lack of food, and survival without any assistance of other bacteria [7] .

Sodium hypochlorite (NaOCL) is the most widely used root canal irrigant. Its superior tissue-dissolving ability and antiseptic qualities make it the best option for nonsurgical endodontic procedures. Higher concentrations of sodium hypochlorite are more effective than 1 and 2 percent solutions and 5.25 % concentration is the most effective[8]. NaOCl, however, has some unfavorable characteristics, including tissue toxicity, the danger of emphysema when overloaded, allergic propensity, undesirable taste and odor, incapability to eliminate the smear layer, and the possibility for mutagenicity of the parachloroanaline product when blended with chlorhexidine (CHX) [9] .

The diode laser is a semiconductor laser that typically combines the elements gallium, arsenide, and additional substances like aluminum and indium to transform electrical energy into light energy. Diode lasers' operating wavelengths don't interact with calcified tissues, making them safe to use for treatments like sulcular debridement, attaining soft tissue excision, hemostasis, and sterilization. Additionally, diode laser therapy is effective at disinfecting root canal walls, particularly lateral dentinal tubules, which are difficult to

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properly reach with traditional treatment. It has an excellent antimicrobial effect because it can penetrate the dentinal tubules up to [10] 1000 μ m deeper than chemical antiseptics, which can only reach 100 μ m. Moreover, it is cost-effective, and its temperature rise has been shown to be within an appropriate limit for permanent teeth [11].

Ozone (an inorganic and allotropic oxygen molecule) is another promising disinfectant agent that has been developed. Ozone in the aqueous phase is easily handled, has a faster antiseptic effect, and can be used as a solution for soaking medical or dental equipment. Compared to other antimicrobial irrigations like sodium hypochlorite, it is greatly biocompatible to human oral cells. At various concentrations, aqueous ozone has a potent bactericidal effect that can effectively kill and eradicate oral-resistant pathogens like *E. faecalis, Candida albicans, and Pseudomonas aeruginosa* [9]. Owing to the increase in bacterial resistance to chemical antimicrobial agents, alternative herbal irrigants have recently been assessed. *Nigella sativa* (NS), also known as a black seed, is gaining popularity as a mystery herb with a strong religious history. Its main bioactive component is thymoquinone. It has antihypertensive, antidiabetic, anticarcinogenic, antiseptic, analgesic, anti-inflammatory, and antioxidant effects [12] .

Dentin permeability may affect the disinfection of the root canal system and the success of the root canal treatment. It is generally assessed by the penetration depth of root canal irrigants into the dentin tubules [13]. The microorganisms can spread inside the dentinal tubules up to 1643 μm making the sufficient disinfection of the root canal system challenging, especially with *Enterococcus faecalis* [14, 15].

Up till now, there have been no studies evaluating Diode laser 635 nm as a root canal irrigant in primary teeth. So, this study aimed to assess and compare the antibacterial efficacy of sodium hypochlorite, diode laser 635 nm, ozonated water, and *Nigella sativa* as root canal irrigants against *E. faecalis* in primary teeth.

2. MATERIALS AND METHODS

2.1. Ethical standards and study setting

A comparative in-vitro study was conducted at the Department of Pediatric Dentistry, Faculty of Dentistry, and the Pharmaceutical Microbiology Department, Faculty of Pharmacy after obtaining the approval of the ethical committee (REC), Faculty of Dentistry (#R-PED-7-21-3). Informed written consent from parents was acquired to use their children's extracted teeth in the research.

2.2. Sample size calculation and randomization

The sample size and power analysis were determined using the Epi-Info software statistical package, version 2002, developed by the World Health Organization and the Center for Disease Control and Prevention in Atlanta, Georgia, USA. Based on the previous study results conducted by Kushwah et al., [8] and assuming an alpha (α) level of 0.05 and power = 80%, the predicted sample size was N=44. The authors had increased the sample size to 50 in each group to compensate for missed data.

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Primary anterior teeth were gathered from the outpatient clinics of the Pediatric Dentistry Department at the Faculty of Dentistry. The study sample was comprised of four hundred and fifty primary anterior teeth extracted due to ectopic eruption of permanent successors, serial extraction, or excessive caries with intact two-thirds of the root length. Teeth with previously pathologic root resorption affecting over one-third of the root or having previous root canal treatment were excluded. Randomization was performed using Random Allocation Software (http://www.randomization.com). An independent investigator handled the randomization procedure, creating a computer-generated randomization list that was preserved in a sealed envelope and then, used to assign teeth that fulfilled the inclusion criteria to one of the study groups.

2.3. Specimen Preparation

The teeth were inspected for an intact root surface, any calculus, debris, and periodontal tissue, which were removed using a curette to obtain a clean external surface. To standardize the root length, 9 mm was measured from the root's apex toward the coronal direction using a digital caliper then the tooth crown was removed with a high-speed handpiece and a coolant water spray. The root specimens were then preserved in saline solution until used to avoid dryness. All root canals were instrumented using circumferential filing movement up to a # 35 K file (Mani Inc., Japan). After each file size, 2 ml of distilled water was used during cleaning and shaping. The apical foramina were then sealed with a flowable composite (Harvard, GmbH, Germany) to prevent the diffusion of microorganisms. Lastly, to clear out any debris, the canals were washed with 5 ml of distilled water. Prior to *E. faecalis* inoculation, all specimens were sterilized in a steam autoclave at 121 °C under 15 psi pressure for 20 min to eliminate all microorganisms.

2.4. *Enterococcus faecalis* culture and inoculation

Bile Aesculin Agar (BAA) (Oxoid, US) was used to selectively isolate and identify strains of *E. faecalis* obtained from the Pharmaceutical Microbiology Department, Faculty of Pharmacy. All microbial procedures were carried out under aseptic conditions. Using inoculating tips, sterilized specimens were injected with an *E. faecalis* clinical isolate cultured in Brain-Heart Infusion (BHI) broth (Difco, Detroit, MI) at 37°C for 24 hours in a 5% CO_2 atmosphere. The organisms were collected by centrifuging at 10,000×g for five minutes, after which were suspended in saline and adjusted with a spectrophotometer to 3×10^6 cells/ml. To allow bacterial growth, specimens were soaked in broth at 37°C. The medium was substituted weekly for four successive weeks. After that, specimens were taken out of the bacterial culture and cleaned with 2.5% sodium hypochlorite solution prior to receiving further treatment. All groups underwent the experimental irrigation procedure by injecting 5 ml of an irrigant solution into the canals using a sterile 20-cc syringe with a 28-gauge needle.

2.5. Experimental groups

E. faecalis-contaminated root canals were divided randomly into seven groups (50 roots/ group) according to different treatment modalities:

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- **Group A**: 2.5 % Sodium hypochlorite (Clorox Co.) (*n*=50): root canals were thoroughly irrigated 3–4 times with 5 ml of 2.5% sodium hypochlorite. Following treatment, the samples were kept in sterile vials.
- **Group B**: Diode laser (Lasotronix, smart M pro 635nm) (*n*=50): root canals were irradiated in a safe mode for 5 seconds, with 10 seconds of resting time (repeated four times) using the Diode laser 653 nm optical fiber which was introduced into the canal to reach the root apex. The treated samples were then placed into sterile vials.
- **Group C**: Ozonated water which was prepared using a Sota water Ozonator generator (model WOZ5) (*n*=50). A 5 ml syringe was used to properly irrigate the root canal three to four times with ozonated water, and the treated samples were then placed into sterile vials.
- **Group D**: *Nigella sativa* oil (Imtenan health shop) (*n*=50): root canals were thoroughly irrigated with ethanolic extract of *Nigella sativa* oil (1:1) three to four times using a 5 ml syringe and the treated samples were then kept in sterile vials
- **Group E**: Diode laser-NaOCl (*n*=50): root canals were filled with 2.5% NaOCl then the optical fiber was introduced 1 mm short of the working length & laser irradiation was performed in a safe mode as group B. 2.5% NaOCl was used as an intermediate irrigant. The treated samples were then kept in sterile vials**.**
- **Group F**: Diode Laser-Ozonated water (*n*=50): root canals were filled with Ozonated water then laser irradiation was performed in a safe mode as group B. As an intermediate irrigant, ozonated water was used. After treatment, the samples were put into sterile vials.
- **Group G**: Diode laser -*Nigella sativa* oil (*n*=50): root canals were filled with ethanolic extract of *Nigella sativa* oil (1:1) then laser irradiation was performed in a safe mode as group B. As an intermediate irrigant, ethanolic extract of *Nigella sativa* oil (1:1) was used. After treatment, the samples were put into sterile vials.

Two Control Groups:

- **Group H**: Negative control (*n*=50): root canals were not contaminated with *E. faecalis* and no treatment with medicament,
- **Group I:** Positive control (*n*=50): root canals were contaminated with *E. faecalis* but no treatment with medicament.

After the disinfection procedures, all samples were washed three times using 1 ml of saline solution to avoid the possibility of irrigant carryover. Paper points # 30 (Dentsply Maillefer, OK, USA) were inserted into root canals at the working length and kept for 60 seconds to absorb the canal contents then, the bacteria were inoculated on blood agar plates. After that, blood agar plates were incubated at 37°C in a CO_2 chamber for 24 hours. A total bacterial count was carried out after complete identification to get the

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CFU/µl then calculated to get the CFU/ml [16]. The laboratory staff and clinicians were blinded to the group assignment during the evaluation of the culture plates.

2.6. Histological teeth staining and sectioning.

The model presented by Galler et al.[14] served as the basis for the staining techniques. To avoid external stains during the experiment, nail polish was applied to the teeth's surface. Then, the canals were filled with 2% methylene blue (CZTL, USA), were dried with paper points and, were let to dry for 72 h. The histological transverse ground sections (100–150 µm) thick were done at the middle third of the root for all teeth according to Maat et al.,[15] schedule. The transverse sections were examined under a light microscope connected to a digital camera (Leica Leica DMS1000). To provide consistency across the images and facilitate accurate measurements, identical objectives were used with fixed resolutions and ideal focus. The images with a standardized scale bar were analyzed with Image J software (National Institutes of Health, Bethesda, MD, USA). After that, two observers blindly assessed the maximum methylene blue penetration depth for all teeth sections in all groups.

2.7. Statistical Analysis

The IBM SPSS version 19 (Statistical Package for Social Studies) created by IBM, Illinois, Chicago, USA was used to organize, tabulate, and statistically analyze the collected data. For *E. faecalis* count and percentage reduction by different treatment modalities, the range, mean, standard deviations, median, and interquartile range were calculated. The differences in bacterial count between the experimental groups and the control group were tested using analysis of variance (ANOVA), while the differences between different experimental groups were tested using the Bonferroni test. The significance level was set at $p<0.05$. For the maximum methylene blue penetration depth and penetration percentage, the Chi-square test was used.

3. RESULTS

The total number of colony-forming units (CFU/ml) obtained from the experimental groups, and the comparison of percentage reduction was presented in Table-1 & Figure-3. Diode laser 635nm showed maximum antibacterial efficacy against *E. faecalis* with the least CFU/ml (83.02+2.00 CFU/ml) compared to *Nigella sativa* (114.62+3.32 CFU/ml), ozonated water (182.24+3.26 CFU/ml) and 2.5% NaOCl (1411+34.58 CFU/ml) groups, respectively, with a highly significant difference ($P<0.0001$). The antibacterial activity of the diode laser-*Nigella sativa* group was the most effective against *E. faecalis* among the laser-combined groups, with the least CFU/ml (49.40+2.94 CFU/ml), followed by diode laser-ozonated water (57.22+3.00 CFU/ml) and diode laser-NaOCl (64.30+2.95 CFU/ml), respectively, with no statistically significant difference ($P > 0.05$).

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Table 1. Bacterial counts (CFU/ml, mean ± SD) and bacterial reductions percentage in the different tested groups.

Group	Bacterial Counts (CFU/ml)			Bacterial Reduction Percentage		
	Range	Mean±SD	Median	Range	Mean±SD	Median
(A)NaOCl	1340-1460	1411±34.58	1410	24-33	27.48±1.87	27.25
(B)Diode Laser	80-86	83.02+2.00	83	95-96	95.73±0.13	95.75
(C)Ozonated-water	178-119	182.24+3.26	182	90-91	90.63±0.23	90.63
(D)*Nigella sativa*	110-119	114.62±3.32	115	93.7-94.5	94.11±0.21	94.11
(E)Diode laser-NaOCl	60-69	64.30±2.95	65.00	96.4-97.0	96.96±0.16	96.65
(F)Diode laser-Ozonated water	52-64	57.22±3.00	57.50	96.8-97.4	97.06±0.16	97.05
(G)Diode laser-*Nigella sativa*	42-54	49.40±2.94	50.00	97.2-97.9	97.46±0.16	97.43
(I)Positive control	1900-2000	1946.60±31.60	1945	-----	-------	------

ANOVA: p <0.001 between all groups. Each group is significantly different from other groups except Diode laser-Ozonated water group is not significant from each of Diode laser-NaOCl and Diode laser-Nigella sativa groups.

The highest percentage of bacterial reduction among the laser-combined groups of this study was 97.46% for the diode-*Nigella sativa* group (group G), with no statistically significant difference from the other two groups (P > 0.05). The mean percentage reduction in CFU/ml in Group F (diode laser-ozonated water) was 97.06%, and in Group E (diode laser-NaOCl) it was 96.96%. There was a highly significant difference in percentage reduction among all single experimental groups (P<0.0001), with the highest percent bacterial reduction (95.73%) for the diode laser group (group B).

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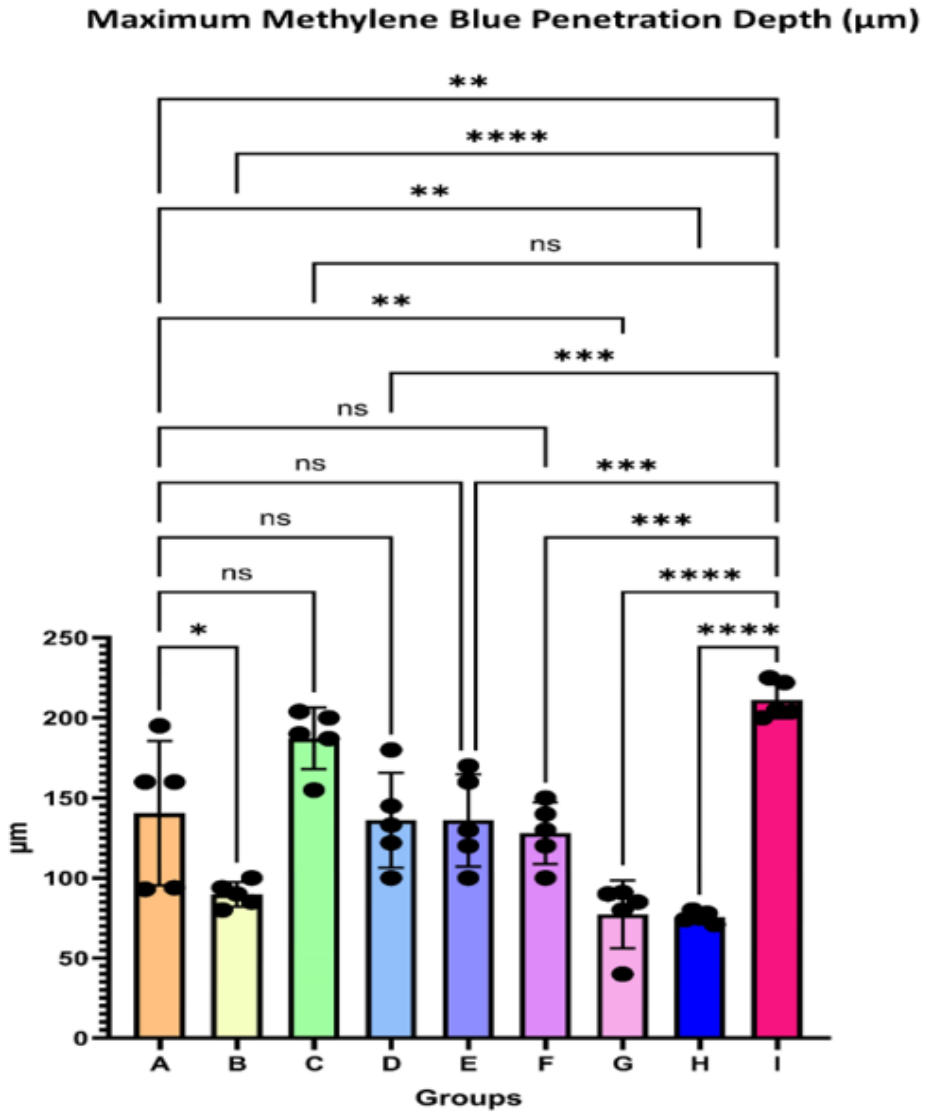

Fig.1: Bar chart showing ANOVA for the dentin permeability of all teeth in all groups.

From the bar chart in the figure. 1; The dentin permeability of the teeth showed the lowest amount in diode laser (group B) among the single experimental groups with a significant difference when compared with NaOCl (group A) followed by the *Nigella sativa* oil (group D) then the Ozonated water (group C) with no significant difference with NaOCl (group A). All the single experimental groups showed significant differences with the positive control (group I) except the Ozonated water (group C). Similarly, the maximum dentin permeability of teeth showed the lowest amount in diode-*Nigella sativa* (group G) among the laser-combined groups with a significant difference with NaOCl (group A) followed by the Diode Laser-Ozonated water (group F) then the Diode laser-NaOCl (group E) with no

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significant difference with NaOCl (group A). All the laser combined groups showed significant differences with the positive control (group I).

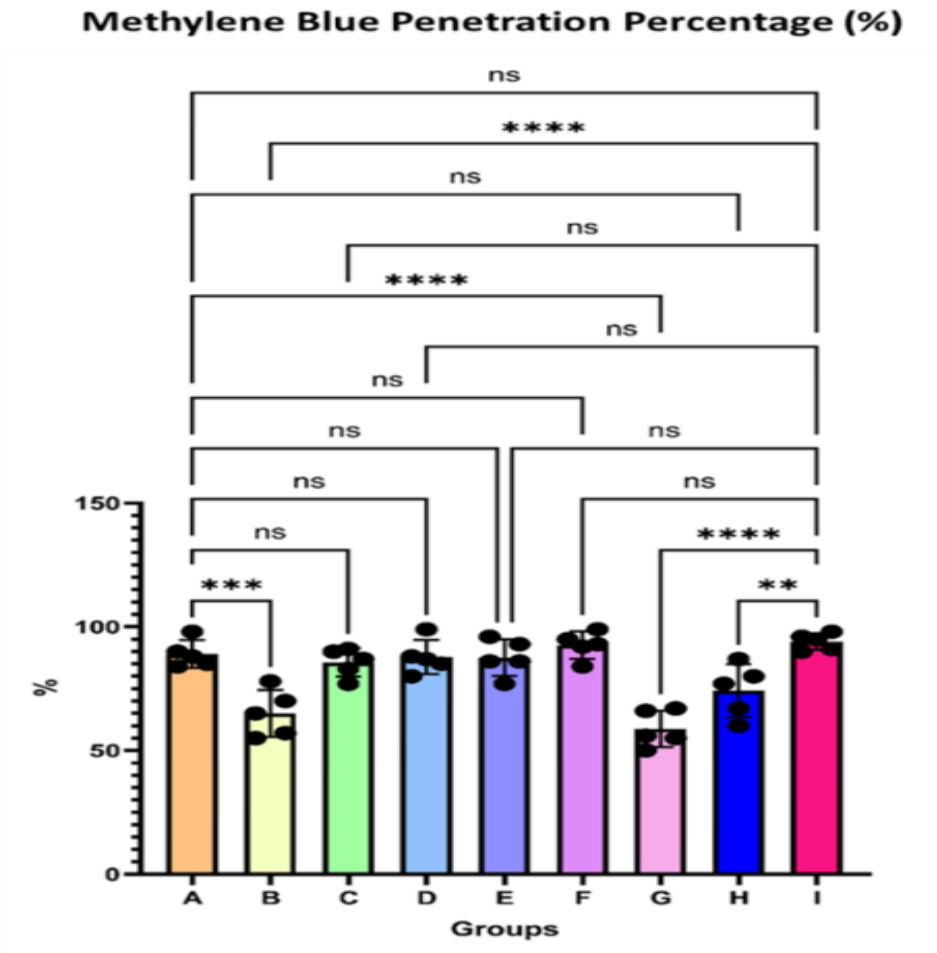

Fig.2: Bar chart showing ANOVA for the dentin penetration percent of all teeth in all groups.

From the bar chart in the figure. 2; The dentin penetration percent of the teeth showed the lowest amount in diode laser (group B) among the single experimental groups with a significant difference when compared with NaOCl (group A) followed by the Ozonated water (group C) then *Nigella sativa* oil (group D) the with no significant difference with NaOCl (group A). All the single experimental groups showed no significant differences with the positive control (group I) except the diode laser (group B). Similarly, the penetration percent of the teeth was the lowest amount in diode-*Nigella sativa* (group G) among the laser-combined groups with a significant difference with NaOCl (group A) followed by the Diode laser-NaOCl (group E) then Diode Laser-Ozonated water (group F) with no significant difference with NaOCl (group A). All the laser-combined groups showed no significant differences with the positive control (group I) except the diode-*Nigella sativa* (group G) which showed a highly significant difference.

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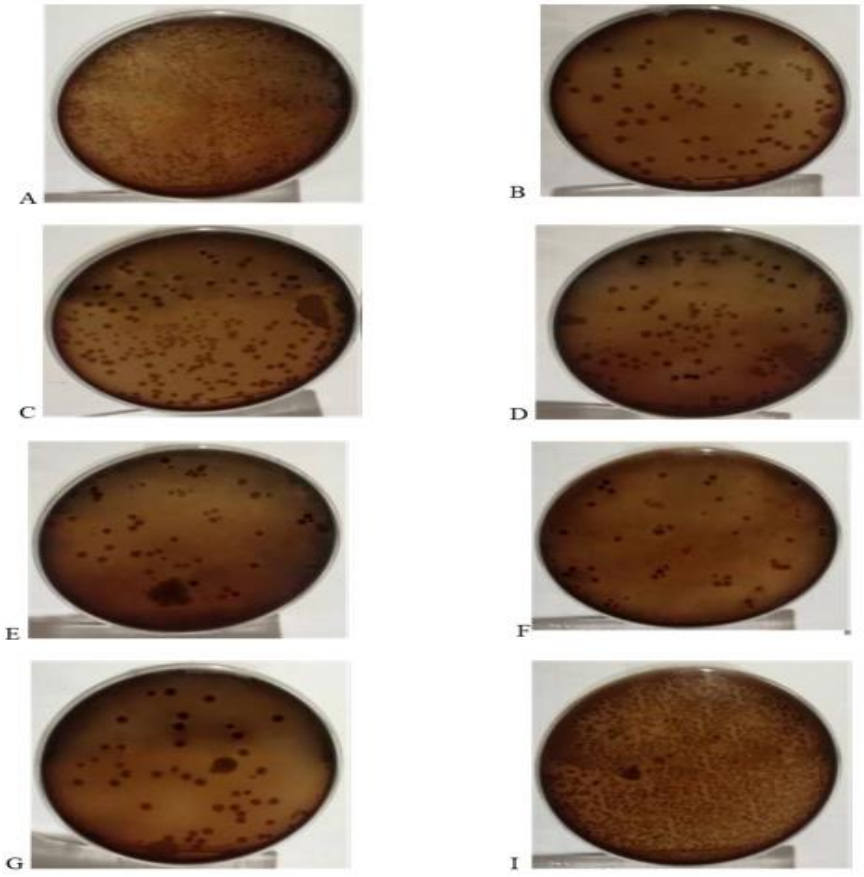

Fig.3: Colony-forming units (CFUs) after treatment: (A) Sodium hypochlorite group; (B) Diode-laser; (C) Ozonated water group;(D) *Nigella sativa* group; (E) Diode laser-NaOCl group; (F) Diode laser-Ozonated water group; (G) Diode laser-*Nigella sativa* group; (I) Positive control group.

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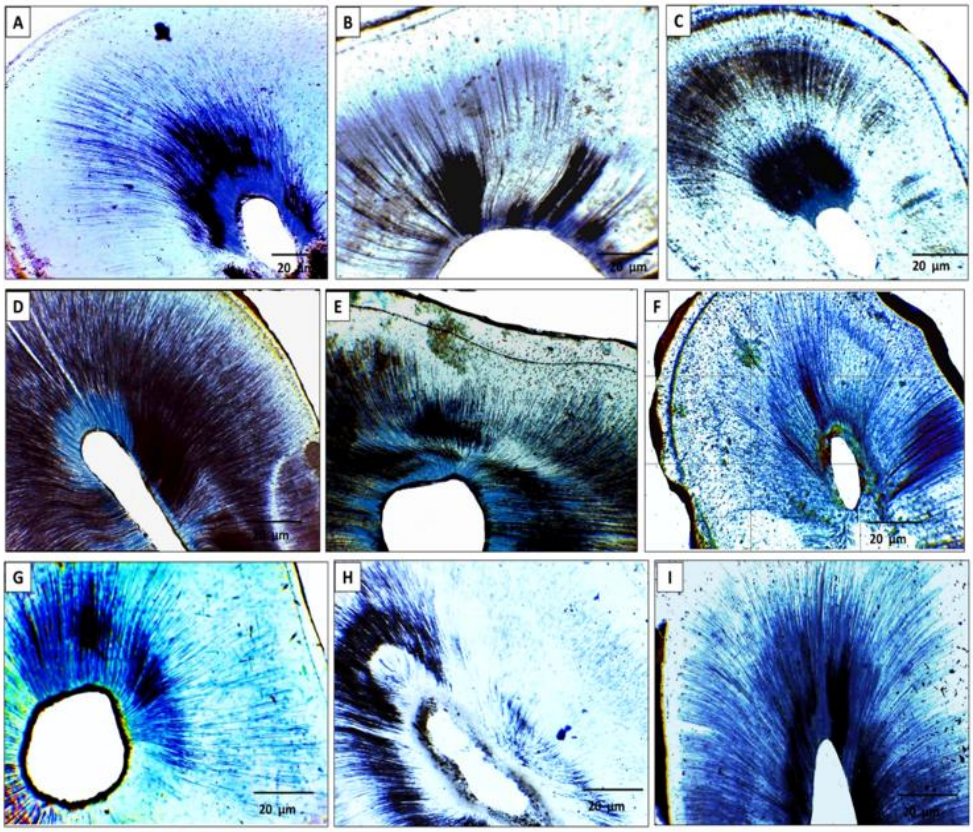

Fig.4: Photomicrographs of methylene blue penetration in T.S ground section for all groups. A: Sodium hypochlorite group, B: Diode laser group, C: Ozonated water group, D: *Nigella sativa* oil group, E: Diode laser-NaOCl group, F: Diode Laser-Ozonated water group, G: Diode laser -*Nigella sativa* oil group, H: Negative control group, and I: Positive control group. (T.S., orig. mag..A-I X 40).

4. DISCUSSION

Primary teeth have unusual internal pulp morphology, including horizontal anastomoses, and inaccessible areas. As a result, endodontic treatment of primary teeth is regarded as highly sophisticated also, selecting an effective antibacterial irrigant is challenging [17].

In this study, *E. faecalis* was selected as the test microorganism because it is one of the most commonly isolated in teeth with pulp necrosis. Also, it is more resistant to starvation, difficult to eradicate and conventional irrigation methods cannot eliminate it; this agreed with Cogulu et al., [6] who found that the most prevalent species of bacteria in primary root canal were *E. faecalis, Porphyromonas gingivalis* and *Treponema denticola*. The incubation time is one of the factors that influences the depth of penetration of microorganisms. In the current study, the teeth specimens were incubated with *E. faecalis* for 24 hours which is sufficient time to infect the dentinal tubules. This is in accordance

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with Behnen et al.,[18] who demonstrated that there is no difference in bacterial penetrations into dentine channels with more incubation time than 24 hours.

In the current study, diode laser 635 nm irradiation was used in a safe mode for 5 s, followed by 10 s of resting time to prevent a temperature rise to an unfavorable level because a temperature rise to a critical point can harm the tissues surrounding the tooth. The periodontal tissues may suffer irreparable damage when the temperature rises by about 10 °C and the treatment lasts for 1 minute. This agreed with Schoop et al., [11] who concluded that a diode laser showed the lowest temperature increase compared with other laser devices. While it disagreed with Dai et al., [19] who irradiated the specimens with the diode laser at an output power of 2.0 W for 5 s and a wavelength of 810 nm in continuous mode. In addition, ethanol was used as a solvent to prepare the ethanolic extract of *Nigella sativa* oil (1:1) to help easy irrigation with the oil also, the ethanol concentration was not increased by more than 50% to avoid the antimicrobial effect of ethanol. This agreed with Neha Jain et al., [12], but it disagreed with the study performed by Al-Badr & Al-Huwaizi [20] in which the *Nigella sativa* oil was mixed with 10% dimethyl sulfoxide (DMSO) solution in 3:1, 1:1, 1:3 and 1:7 (oil: 10% DMSO).

The results of the current study showed a highly significant difference between 2.5% NaOCl, ozonated water, *Nigella sativa,* and diode-laser groups ($P<0.0001$), and the lowest residual bacterial load was observed in the diode laser group. This agreed with Mathew et al.,[21] who concluded that the diode laser group showed better antibacterial efficacy and least viable bacteria when compared to conventional needle irrigation, PIPS, and Endo Activator groups. The excellent microbicidal effects of diode laser irradiation may be related to its greater penetration depth (up to 1000 μm into dentinal tubules) compared to chemical disinfectants, which have a restricted penetration power of 100 μm. In contrast to our results, Öter et al.,[22] reported that 2.5% NaOCl exhibited the highest antibacterial effect than diode-laser, Endosafe, photo-activated disinfection, and ozone groups.

According to the findings of this study, the diode-laser group had the highest percent reduction of colony-forming units followed by *Nigella sativa*, ozonated water, and NaOCl respectively with highly significant differences ($P<0.0001$). The lowest percent reduction of NaOCl can be thought to be due to its restricted dentine penetration (about 130 μm) to infiltrate and disinfect the deeper dentinal tissues. This was in accordance with the study performed by Jambagi et al.,[23] study in which the diode laser group showed the highest reduction of the microbial count with 60.92% disinfection compared to 37.97% reduction with 2.5 % NaOCl group. In contrast, it disagreed with Sohrabi et al.,[24] who demonstrated that NaOCl resulted in 99.87% removal of the bacteria compared to the 980-nm diode laser which led to 96.56% bacterial reduction. This conflict may be attributed to differences in NaOCl concentration, as well as differences in physical characteristics (wavelength, peak power, and mode) and emission tips between the laser radiations used.

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The present study results of the *Nigella sativa* group showed a promising antibacterial activity with decreased bacterial count compared to the 2.5 % NaOCl group. These results are in accordance with Neha Jain et al., [12] who compared the antibacterial potency of *Nigella sativa* oil against *E. faecalis* to 2.5% sodium hypochlorite and concluded that *Nigella sativa* oil has bactericidal effects by 30 mins of its exposure to *E. faecalis* while 2.5% NaOCl showed reduced growth of *E. faecalis* at the end of two-hour exposure. Also, this was in coincidence with Al-Badr & Al-Huwaizi [20] who tested the sensitivity of *E.faecalis* to different oil concentrations and reported that *Nigella sativa* oil showed large inhibition zones when compared to calcium hydroxide, tea tree oil, and thyme oil. The presence of other volatile oils in the chemical constituents of the *Nigella sativa* oil such as nigellone, thymoquinone, thymol, carvacrol, α & β-pinene, d-limonene, d-citronellol, p-cymene and 2-(2- methoxy propyl)-5-methyl-1,4-benzenediol, could explain its antimicrobial effect.

The ozonated water group in the current study results had comparable antimicrobial effectiveness in relation to the positive control group (2.5% NaOCl) with a decreased bacterial count. This agreed with Goztas et al.,[25] who investigated the antimicrobial properties of ozonated water, ozonated water with ultrasonication, 2.5% NaOCl, and 2 % chlorhexidine (CHX) in primary root canals contaminated with *E.faecalis* and concluded that ozonated water was effective compared with positive control after 6 hours. In contrast with the present study results, Can et al., [26] tested the synergetic antibacterial effect of gaseous ozone and ozonated water against *E. faecalis* in human root canals and revealed that the combination of gaseous ozone and ozonated water showed significantly less antimicrobial effect when compared with NaOCl and CHX.

The synergetic effect of diode laser 635 nm was evaluated on the tested irrigant solutions in the present study; it was found that diode laser combined with other irrigants had a superior bactericidal effect than diode laser alone. This is explained by the fact that laser energy tends to press irrigating solution faster and deeper into complex canal anatomy. These results were in line with the study conducted by Vaziri et al., [27] which concluded that the combination of diode laser 625 nm and 2.5% NaOCl achieved maximum reduction in recovered viable bacteria. Also, it was in accordance with Dai S et al., [19] who revealed that the use of an 810 nm diode laser, particularly in conjunction with 5.25% NaOCl, was more effective for disinfecting infected primary tooth root canals than 5.25% NaOCl alone. Moreover, it was in agreement with Kushwah et al., [8] who found that the least mean CFU/mL was seen in the diode laser 980 nm-ozonated water group (mean rank score of 20.75) compared to the Ozonated water group (mean rank score of 105.37).

The highest percent reduction among the laser-combined groups of this study was 97.46% for the diode-*Nigella sativa* group with no statistically significant difference with the other two groups. In addition, the diode-NaOCL group showed a promising percent reduction; these findings are consistent with Sarda et al., [28] who concluded that a significant reduction (98%) in the *E. faecalis* count was observed when the NaOCl was used in combination with the diode laser. Furthermore, Preethee et al.,[29] reported a superior bactericidal effect of 908 nm diode laser used in conjunction with conventional

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chemo-mechanical techniques (5.25% NaOCl, 17% Ethylenediaminetetraacetic acid (EDTA); 1.3% NaOCl, MTAD; and 8.5% saline) with a significant elimination of *E. faecalis* in the apical third of root dentin. This could be attributable to a fiber optic tip associated with a diode laser, which allows for greater access to previously inaccessible parts of the tubular system, resulting in an excellent bactericidal effect in the root canal dentin.

The dentin permeability and penetration percent of the teeth showed the lowest amount in diode laser (group B) among the single experimental groups with a significant difference when compared with NaOCl (group A). Also, all experimental group showed low dentin permeability and penetration percent when compared with the positive control group. this was agreed with Ren et al., [30] who performed the first study regarding to the NaOCl penetration when there were clinical conditions that allowed deep penetration of the bacteria and their by-products into the dentinal tubules of dentine. However, in a histological study done by Ricucci et al., [31] they found that deep crown caries and pulpitis cause reparative dentine formation on the canal walls that might decrease dentine permeability as confirmed by Ren et al., [30]. Other studies concluded that a tubular histological type of tertiary dentin was the cause of the penetrability of root dentin as it inhibited the endodontic irrigants penetration into the root canal system [31, 32]. One of the study's limitations was the difficulty of collecting the appropriate teeth sample (teeth with intact two-thirds of the root length).

CONCLUSION

Depending on the present study results, it was concluded that the diode laser 635 nm showed better antibacterial efficacy, especially the diode-*Nigella sativa* group, which revealed satisfactory bactericidal effects in experimentally infected primary teeth's root canals. The use of a diode laser in conjunction with *Nigella sativa* oil may be the best protocol to increase the therapeutic success rate and can offer useful recommendations for clinical usage in primary endodontic treatment. Future research is encouraged to determine the best laser type, settings, application technique, and irrigant to sync with lasers to achieve more effective root canal disinfection.

Bullet points

- Root canal irrigants play a significant role in pediatric endodontics due to the peculiar internal configuration.
- *Enterococcus faecalis*, one of the most common bacteria in recurrent root canal infections, and it causes four to forty percent of all primary endodontic infections.
- 635 nm diode laser and Nigella sativa oil can eliminate and disinfect *E. faecalis* from primary root canals.

Ethics approval and consent to participate.

Ethical Approval for this study was obtained from the ethical committee (REC), Faculty of Dentistry, Tanta University (#R-PED-7-21-3). All our methods were performed in

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accordance with its relevant guidelines and regulations. Informed written consent from parents was acquired to use their children's extracted teeth in the research.

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Authors' contributions

S.M.H.: participated in the study design, collected the data, and drafted the manuscript; **W.Y.A.:** participated in the study design, data collection, and drafted the manuscript**. S.S.E.**: participated in the study design, data collection, and drafted the manuscript; **M.M.E**: participated in the study design, collected the data, and revised the manuscript; **I.A.K**: participated in the study design, performed statistical analyses and interpretation of the data; All authors read and approved the final manuscript.

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