Original Article

Antimicrobial Effect Of Diode Laser With Curcumin Nanoparticles Against Enterococcus Faecalis. (In Vitro Study)

Rania A. Ibrahim [1], Ahmed A. Zaky [2], Tarek A H. Harhash[3], Marwa M. Abdelgwad[4]

1. M.Sc. in Endodontics, Faculty of Oral and Dental Medicine, Cairo University, Egypt.
2. Professor of laser applications in oral and dental medicine and head of department of medical Laser Applications, National Institute of Laser Enhanced Sciences (NILES), Cairo University, Egypt.
3. Professor of dental laser applications, Medical Laser Applications Department, National Institute of Laser Enhanced Sciences (NILES), Cairo University, Egypt.
4. Assistant professor of medical biochemistry and molecular biology, Faculty of Medicine, Cairo University, Egypt.

Email: raniashehata@gmail.com
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Abstract

Aim: to determine the bactericidal effect of diode laser (445 nm) and curcumin nanoparticles on Enterococcus faecalis. **Methodology:** Fifty extracted maxillary anterior human teeth with one root canal were inoculated with Enterococcus faecalis and organized into five groups; (I) Control group, (II) Sodium hypochlorite group, (III) Laser group, (IV) Curcumin nanoparticles group and (V) Laser with curcumin nanoparticles group. After the treatment protocol, the samples were analyzed by colony forming units (CFU) for analysis of the remaining bacteria. Also, they were stained with dyes to detect live and dead bacteria using a confocal laser scanning microscope (CLSM). Statistical analyses were performed using using ANOVA test followed by Tamahne post hoc test for pairwise comparisons. Significance level for statistical tests was set at 0.05. Statistical analysis was performed using SPSS software (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) **Results:** CFU: The control group showed the highest bacterial load followed by the CurNP group then the CurNP-L group then the laser group and finally the NaOCl group with a significant difference between them. CLSM: The CurNP-L group showed the highest bacterial percentage reduction followed by the NaOCl group then the laser group then CurNP group and finally the control group with a significant difference between them. **Conclusion:** Curcumin nanoparticles had an antibacterial effect on E.feacalis which could be enhanced using low power diode laser.

Key Words: Enterococcus faecalis, NaOCl, CurNP, diode laser

INTRODUCTION

The goal of a root canal treatment is to remove bacteria and its byproducts while also preventing re-infection of the root canal system after mechanical disinfection. [(1)] Unfortunately, it was discovered that using the conventional methods was insufficient for completely cleaning the root canal system, making its accomplishment more challenging. [(2-4)] Because irrigating solutions don't penetrate far enough, they can't completely kill all the microorganisms in the deeper layers. [(5)] Though, dentists have been using diode lasers for a variety of dental applications, with positive outcomes for teeth disinfection. [(6-8)]

Due to the presence of endogenous photosensitizing chromophores in pathogenic microbes, blue light in the 400–470 nm range of the spectrum is currently becoming more widely recognized as a phototherapy–based antimicrobial agent with significant antimicrobial activity against a wide range of bacterial and fungal pathogens. Additionally, it is less likely to give rise to resistance formation than antibiotics and less harmful to host cells than ultraviolet radiation. [(9-11)]

The widely recommended root canal irrigant is sodium hypochlorite due to its effectiveness in eradicating microorganisms and ability to dissolve tissue. [(12)] However, because it may result in tissue destruction of the tissues, direct application of sodium hypochlorite may be hazardous to the host. [(13,14)]

Enterococcus faecalis which is a normal inhabitant of the oral flora and a Gram-positive facultative anaerobic microorganism, is linked to endodontic treatment failure as well as in periradicular lesions including primary and secondary endodontic infections. E. faecalis is discovered in 40% of primary endodontic infection cases, while it is more frequently discovered in 67–77% of secondary periradicular infection cases. [15,16] Therefore, Enterococcus faecalis is the most prevalent bacteria causing root canal treatment failures. [17-19] It is resistant to antibacterial drugs like NaOCl in various treatments. [20,21] Due to biofilm production and penetration into the dentinal tubules, it is tolerant to calcium hydroxide pastes and alkaline pH, which generally suppress other bacteria. [22,23]

The use of photodynamic therapy in the management of localized bacterial infections has demonstrated promising applications. [24] It promotes the production of reactive oxygen species, including hydroxyl radicals, superoxides, and singlet oxygen, by photoactivating a photosensitizer with low-energy coherent or non-coherent light while oxygen is present. These reactive oxygen species interact with numerous receptors in a bacterial cell, causing the target cells to disintegrate. Although both gram-positive and gram-negative bacteria have been proven to be susceptible to this treatment, the gram-positive bacteria are more affected. [25]

The use of natural resources for dental care is now increasing. In India, China, and Southeast Asia, turmeric (Curcuma longa) is widely used as a spice, food preservative, and coloring agent. It has been employed in conventional medicine to treat a variety of diseases. The main yellow bioactive component of turmeric, curcumin (diferuloylmethane), has been found to have a wide range of biological effects, including antibacterial, anti-inflammatory, and antioxidant activity. [26-28] When applied as a photosensitizer, curcumin, a phenolic molecule with an absorption peak at 430 ± 20 nm [29] can produce photodynamic effects that increase the effectiveness of its antibacterial properties. [30]

The objective of this study was to determine the bactericidal effect of low power diode laser 445 nm and curcumin nanoparticles on Enterococcus faecalis.

Aim

To compare the photodynamic therapy of low power diode laser 445 nm with curcumin nanoparticles against Enterococcus faecalis.

Materials and Methods

1. **Samples selection:**
   A total of fifty extracted maxillary anterior human teeth with one root canal were collected. All roots were intact with mature apices.

2. **Samples preparation:**
   The teeth were scaled with ultrasonic scaler to remove all adhering soft tissues and kept in saline solution at room temperature until time of use. Then, they were decapitated at the level of cemento-enamel junction using high speed diamond disc to facilitate the mechanical preparation of the root canals, which the working length was established by subtracting 0.5 mm from the apical foramen and was standardized at 15 mm. The canals were instrumented using ProTaper Universal rotary files.

   ProTaper Universal NiTi rotary file system was used to prepare the canals in a crown-down fashion. The instruments were used in a 16:1 gear reduction handpiece powered by a torque-speed controlled endodontic motor electric motor at a speed of 250 - 300 rpm. The torque was adjusted for each file as recommended by the manufacturer; 3.5 Ncm for SX and S1, 1.2 Ncm for S2, 2.0 Ncm for F1and 2.6 Ncm for F2, F3, F4 and F5.

   Ethylene Diamine Tetra Acetic acid (EDTA) 17 % paste was used as a lubricant during the mechanical preparation to remove smear layer. The irrigation protocol after each file was using 3ml for 1 min 5.25 % sodium hypochlorite (NaOCl) solution filled in a plastic syringe with a side-ended 27-gauge needle inserted passively as deep as possible inside the canals. Then, 3 ml of EDTA solution and a final irrigation with 3 ml distilled water.

   After the completion of the shaping procedure, the apical foramina were sealed with glass ionomer cement from the external surface to prevent loss of solutions. The external roots surface was sealed with a layer of colorless varnish sealing the lateral canals ends and to avoid external microbial contamination.

3. **Samples sterilization:**
   Each root was placed in a micro-tube containing sterile brain-heart infusion (BHI) broth and then all samples were autoclaved at temperature of 121 C for 20 minutes to remove all pre-existing bacteria.
   One root was randomly selected as the negative control which was placed in an incubator for 2 weeks to verify no bacterial growth.

4. Bacterial culture:

The Enterococcus faecalis (ATCC 29212) (E. faecalis) was maintained in culture on K-F Streptococcus agar plates and incubated at 37 °C for 24 h. Isolated colonies of pure cultures were scraped from agar plates by using sterile swab, then suspended in Brain Heart Infusion (BHI) Broth (oxoid, England) and dispersed by vortex under laminar air flow to adjust turbidity of the bacterial suspension. (Macferalad 0.5)

5. Root canals contamination and biofilm formation:

E. faecalis bacterial suspension was placed into all the root canals, each placed into a new micro-tube containing BHI broth and stored at 37 C for 2 weeks. During this period, the intra-canal suspension was replaced with 20 ml of a new suspension every 48 hours **(Fig.1)**.

When the specimens' contamination period had elapsed, one root among the samples was randomly served as the positive control and processed for colony forming units (CFU) analysis to confirm bacterial biofilm formation in root canal system on Congo Red Agar (CRA) **(Fig. 2)**. The agar plates were incubated at 37 C for 24 hours in anaerobic condition.

While the rest of the roots were dried from the BHI broth to receive the laser fiber optic and the antimicrobial solutions using a sterile dried paper points size 50 which were used once in each root.

Fig (1): Some of teeth samples after incubation.

Fig (2): Positive control CFU.

6. Root canals classification:

The samples were randomly divided into five main groups:

Group 1: Control

Group 2: Sodium Hypochlorite (NaOCl)

Group 3: Laser

Group 4: Curcumin nanoparticles (CurNP)

Group 5: CurNP + Laser

Sodium Hypochlorite group:

It is conventional antibacterial debridement using 5 ml of 5.25% NaOCl irrigation for 1 minute with 27-gauge side end needle plastic syringe that was introduced passively up to 1 mm from the working length. It was injected to fill the canal and left for 1 min.

Low power diode laser group:

Pioon diode laser device **(Fig. 3)** was used with the 445 nm wavelength, output power of 0.1 W. The fiber optic tip of 200 microns diameter and 3 cm long. Its movement was in a spiral way from coronal to apical and vice versa, 10 movements / min, by the same operator, starting from 1 mm short of the working length, in a continuous mode and with exposure time for 1 min. Decontamination of the optical fiber tip was done after each root canal by a piece of gauze.

Fig (3): Pioon dental laser device.

Curcumin nanoparticles (CurNP) group:

The study used their particle size of < 50 nm and concentration of 200 ppm. CurNP **(Fig.4)** were injected to fill the canal, left for 5 min and covered by aluminum foil in the dark.

Fig (4): Curcumin nanoparticles colloidal solution.

7. **Samples after disinfection**:
   The canals were irrigated with 5ml of distilled water to wash out used solutions, dried with sterile paper point and then were placed again in sterile micro-tubes filled with BHI broth and stored in an incubator at 37 C.

8. **Microbial analysis (CFU):**
   To assess the remaining root canals contamination by Enterococcus Faecalis, the BHI broth inside all the canals was dried using sterile paper points and the canals were filled with sterile normal saline solution as a transfer fluid. The collection was made immediately after the treatment using sterile paper points size F5 left in the root canals for 1 minute. The paper points used were transferred to tubes with 1 ml of sterile saline solution and agitated in vortex mixer (Vortex AP 56, Phoenix, Araraquara, SP,Brazil) for 1 min and afterwards, serial decimal dilutions were made and seeded on petri dishes with CRA **(Fig.5)**. The plates were incubated under microaerophilic conditions at 37 ° C for 48 h. The results were obtained by means of counting colony forming units per milliliter (CFU mL−1).

Fig (5): Congo Red Agar with swab.

9. **Confocal Laser Scanning Microscope (CLSM):**
   The samples of CLSM were sectioned for detecting viable and dead bacteria on root canals walls. The sectioning was done precisely into two halves by IsoMet device **(Fig. 6).** Then, each half was stained by acridine orange (AO) and propidium iodide (PI) dyes respectively just before scanning **(Fig. 7).**
   CLSM was used to emit 40x objectives for scanning. Multiple random areas all over the root canals were scanned. The images taken for the median intensity of green (live with intact cell membrane) and red (dead with damaged cell membrane) bacteria and was calculated by a specific software.

Fig (6): IsoMet device

Fig (7): Confocal Laser Scanning Microscope (CLSM)

10. **Statistical Analyses:**
    Data were presented as mean and standard deviation. Between group comparisons were conducted using ANOVA test followed by Tamahne post hoc test for pairwise comparisons. Significance level for statistical tests was set at 0.05. Statistical analysis was performed using SPSS software (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.)

Results

1. **CFU:**
   The control group showed the highest bacterial load followed by the CurNP group, then the CurNP-L group then the laser group and finally the NaOCl group. ANOVA test showed a significant difference between the five groups (p<0.001). **(Table 1) (Fig 8 & 9)**.

Pairwise comparisons:
Tamahne post hoc test showed no significant difference between the CurNP group and the CurNP-L group. There were significant differences in bacterial loads between all other pairs of groups.

Table (1): mean, standard deviation (SD) and the results of ANOVA test and Tamahne post-hoc test for comparison of bacterial loads in CFUs/ml between the five groups:

	CurNP-L group	**CurNP group**	**Laser group**	**NaOCl group**	**Control group**	***p* - value**
Mean	932^{b}	1080^{b}	529^{c}	39^{d}	4510^{a}	<0.001*
SD	277.3	297.4	41.8	12.9	604.5	

*Significant at p<0.05
**different lower-case letters indicate statistical significance between each group.

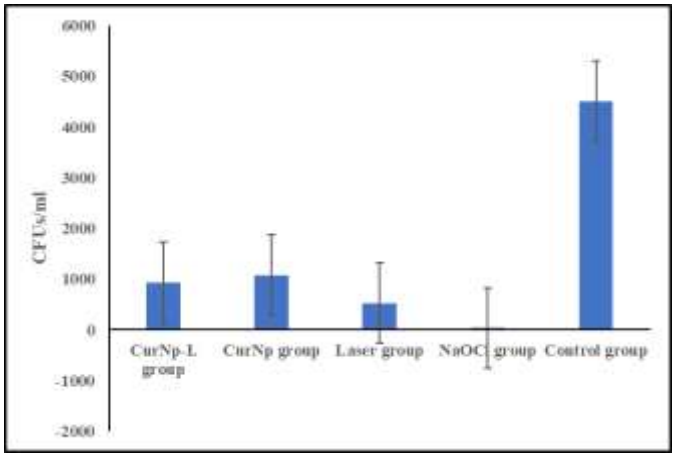


Fig (8): Bar chart representing the mean bacterial load in the 5 groups.

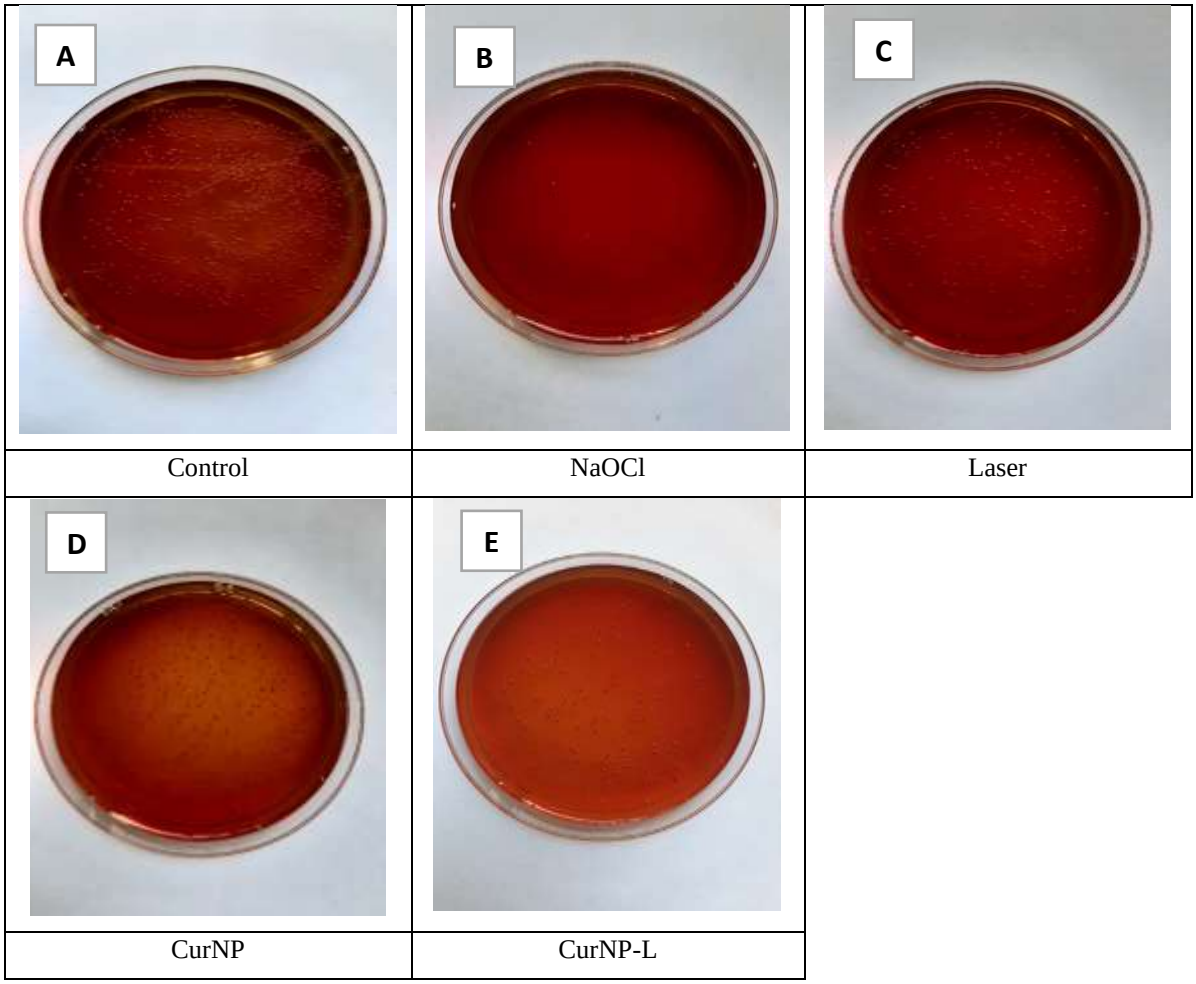


Fig (9) Colony forming units agar plates showing E.faecalis. **(A)** Control group. **(B)** NaOCl group. **(C)** Laser group. **(D)** CurNP group. **(E)** CurNP-L group.

2. CLSM measuring bacterial percentage reduction:

The CurNP-L group showed the highest bacterial percentage reduction followed by the NaOCl group then the laser group then CurNP group and finally the control group. ANOVA test showed a significant difference between the five groups (p <0.001). **(Table 2) (Fig 10 & 11)**.

Pairwise comparisons:
Tamahne post hoc test showed no significant differences in bacterial percentage reduction between the CurNP-L group, the laser group and the NaOCl group. There was no significant difference between CurNP group and the control group. There were significant differences between all other pairs of groups.

Table (2) mean, standard deviation (SD) and the results of ANOVA test and Tamahne post-hoc test for comparison of bacterial percentage reduction between the five groups:

	CurNP-L group	**CurNP group**	**Laser group**	**NaOCl group**	**Control group**	***p - value***
Mean	49.1%[a]	37.3%[b]	47.3%[a]	47.7%[a]	33.9%[b]	<0.001*
SD	10.5%	12.4%	5.0%	8.9%	4.0%	

*Significant at p<0.05
**different lower-case letters indicate statistical significance between each group.

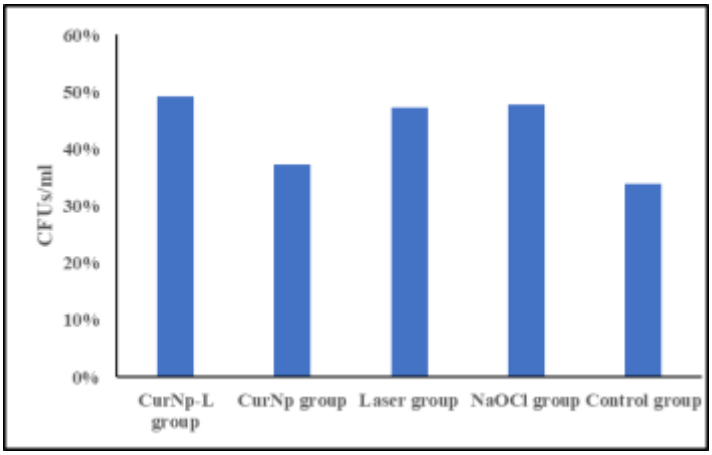
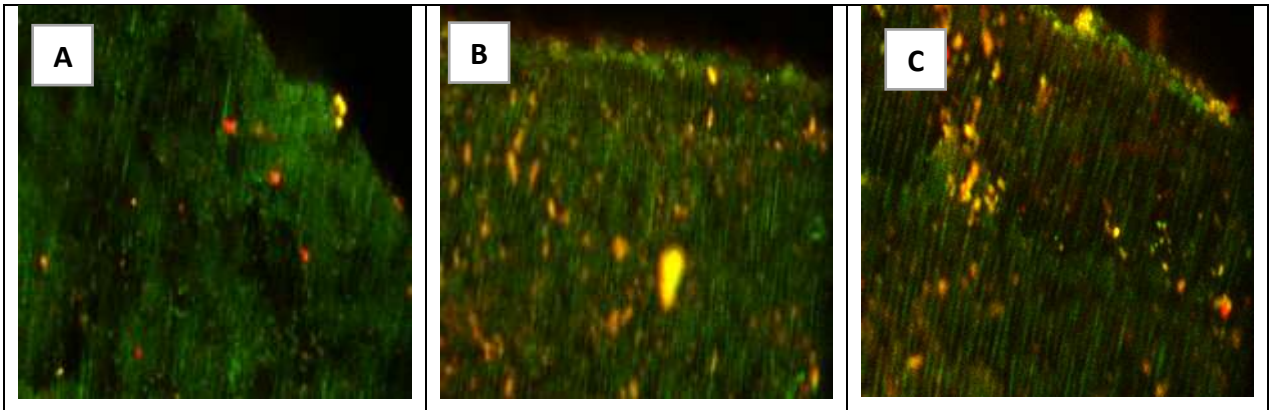
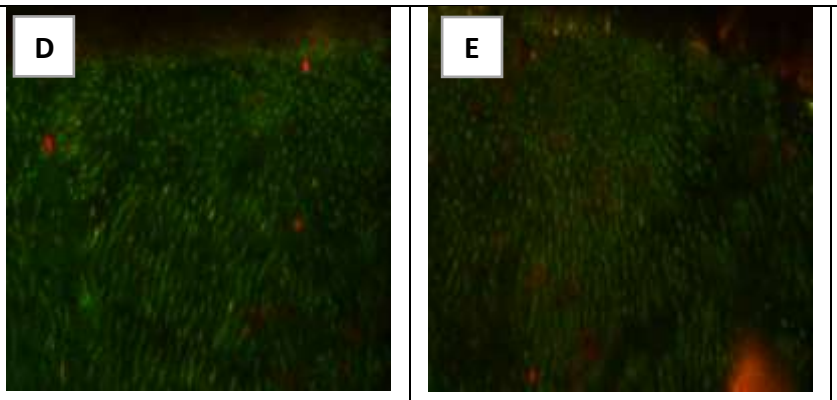


Fig (10): bar chart representing the mean bacterial percentage reduction in the 5 groups.





CurNP	CurNP-L

Fig (11) Colony forming units agar plates showing E.faecalis. **(A)** Control group. **(B)** NaOCl group. **(C)** Laser group. **(D)** CurNP group. **(E)** CurNP-L group.

Discussion

The purpose of this study was to test the antimicrobial effect of low power diode laser 445 nm with silver nanoparticles against Enterococcus faecalis. That is the oral cavity's most resilient species leading to root canal treatment failure. [31] It possesses virulence-enhancing substances such enzymes, cytolysin and lipoteichoic acid. Also, the fact that E. faecalis enters dentin tubules profoundly may help to explain why they remained resistant following traditional endodontic therapy. [32] It was chosen for this investigation because it has a history of exhibiting antimicrobial resistance to a wide range of medications and has been linked to resistant apical periodontitis. [33]

Sodium hypochlorite was chosen for the main reference since it is the universal optimum effective irrigant for root canal therapy. It can dissolve organic debris, get rid of biofilm and the remnants of necrotic tissue. [34] Hypochloric acid is thought to be the cause of the sodium hypochlorite's action on inorganic material because it combines with insoluble proteins to produce soluble products that make it possible to remove the superficial smear layer. [35] Nevertheless, sodium hypochlorite harms the periapical tissues and decreases the elastic modulus and flexural strength of dentine.

The use of a diode laser for dentinal disinfection has yielded encouraging results. As a result, the 445 nm diode laser has been used in the current investigation to attack E. faecalis. Because to its inherent antibacterial action, which does not require the introduction of exogenous photosensitizers as in photodynamic treatment (PDT) and is less harmful to mammalian cells than ultraviolet irradiation, blue light has gained increasing attention. [36] In addition, methicillin-resistant S. aureus infections, periodontal disorders, and caries have all been successfully treated with that blue light (450–500 nm). [37-39] Blue light alone has been proven to have antibacterial effects on bacteria that cause porphyrin production, including F. nucleatum and P. gingivalis, as well as oral black-pigmented bacteria in dental plaque. [40] Reactive oxygen species (ROS), which are considered even more effective than the oxidant chemicals employed for dental disinfection, formed when some photosensitizers are triggered by blue light, according to reports. [41]

The effective delivery of laser light to the root canal to aid in the reduction of bacterial contamination is made possible by the small diameters of optic fibers (200-320 m). [42] Light was applied by spirally inserting the fiber optic up to the working length in both the cervical-apical and apical-cervical directions. This movement was performed roughly 10 times per minute by the same operator. [43, 44]

Curcumin was selected as the photosensitizer because of its wide absorption band, which spans 400–500 nm and peaks at 430 nm. It naturally contains rhizome Curcuma longa which has an effective antimicrobial impact on bacteria, viruses, and fungi. [45,46] The main benefits of utilizing herbal substitutes include their availability, affordability, longer shelf life, low toxicity, and absence of microbial resistance. [47] However, sodium hypochlorite harms the periapical tissues toxically and decreases the elastic modulus and flexural strength of dentine. [48]

Pre-irradiation period was 5 minutes, during which the curcumin solution was injected into the root canal. [49,50] This time frame is required to allow curcumin to interact with the bacterial wall and enter the dentinal tubules. [51-53] Although that prolonged exposure time employed could be a challenge for clinical use. [54]

The Agar diffusion test is the most used method in vitro for assessing antimicrobial efficacy of different irrigants along with laser irradiation. The results of microbiological cultures demonstrate a positive bactericidal effect of CurNP solution as an endodontic irrigation on E.faecalis, but was less significant than that occurred with CurNP-L and NaOCl at 5.25 % concentration respectively. That agreed by Neelakantan et al study [55], which showed that NaOCl 3% concentration had maximum bacterial activity against E.faecalis biofilm followed by Curcumin. Although it has been speculated that sodium hypochlorite, which only penetrates dentin to a depth of around 100 microM while, aqueous solutions of photosensitizers may diffuse more deeply into infected dentin due to their lower surface tension. [56]

On the other hand, another study showed that there was a significant reduction in the amount of biofilm produced by E.faecalis after using NaOCl 5.25 %, Curcumin activated by LED 450 nm and ICG activated by diode laser 810 nm, without significant difference between them. [49]. Although, these results disagreed with another study resulting that blue LED light activation of Curcumin produced higher antibacterial activity than NaOCl 3% irrigation. [57]

It has been suggested that curcumin induces hydrogen peroxide as an intermediary when illuminated and that this intermediate is harmful to bacterial cells. Curcumin's capacity to have lethal effects without attaching to or being close to germs is a significant benefit [58]. Nonetheless, a photosensitizer's attachment to bacterial cell walls may make the germs more sensitive to light [59].

Also, other studies found that lower significant bacterial count after their exposure with LED blue light with Curcumin than LED alone. [50,54,60] Agreed with Oda et al [50] which suggested that curcumin with LED curing light treatment was as effective as the standard PDT performed with diode laser with methylene blue and have a significant antimicrobial action more than curcumin or LED alone respectively. These results agreed with previous studies. [60-63] As well as Devaraj et al [64] study showed that LED photo-activated curcumin demonstrated superior antibiofilm and antibiotic activities against E.feacalis than triple antibiotic paste, without statistically significant difference .

So, there are many factors, including technical issues, concentration, research type, use of planktonic or biofilm forms, duration, length of exposure, kind of photosensitizer, and many more, that can be used to explain variations between studies [65].

Conclusion

Curcumin nanoparticles had an antibacterial effect on E.feacalis which could be enhanced using low power diode laser 445 nm. This is considered as an adjunctive endodontic disinfection to using the traditional sodium hypochlorite irrigation.

Declarations

Consent for Publication: I certify that the work has been approved by all the authors for submission.
Competing interests: none
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