Evaluation of the Antibacterial Efficacy of Chlorhexidine and Nanocurcumin with and Without Laser (An *In-Vitro* Study)

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Abstract:

Introduction: The aim of the study was to compare the antibacterial efficacy of 0.125% chlorhexidine (CHX) and nanocurcumin when used alone and in combination with a 980 nm diode laser against *Enterococcus faecalis* biofilm. **Materials and methods:** Forty eight extracted human single rooted teeth were selected. Their crowns were resected at the cementoenamel junction (CEJ), and their roots were mechanically prepared. They were sterilized and then infected with *Enterococcus faecalis* for three weeks. They were divided randomly into four groups in accordance with the type of irrigant used. Group (A), normal saline. Group (B), 0.125% CHX. Group (C), nanocurcumin irrigant. Group (D),nanocurcumin irrigant and a 980 nm diode laser. The antibacterial efficacy was evaluated before and after irrigation. **Results:** The reduction percentage was 93.5% in group (B), 90.26% in group (C), 99.5% in group (D), and 0.15% in group (A). **Conclusion:** The combination of nanocurcumin irrigant and a 980 nm diode laser is a promising modality in elimination of *Enterococcus faecalis* completely from the root canals.

Keywords: Biofilm, Chlorhexidine, Curcumin, Diode laser, *Enterococcus faecalis*.

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Introduction:

The success of root canal treatment relies on the total elimination of pathogenic microorganisms from the root canals. [1] Because they were considered the main causative factor of endodontic failures and periapical inflammations, especially biofilms, as they have high impedance to antibacterial agents. [2,3]

*Enterococcus faecalis*is a facultative anaerobic bacteria that is detected in cases with secondary infections and endodontic failures because it can live without nutrition for a long period of time and can penetrate deeply into dentinal tubules. [4, 5]

Traditional endodontic treatment was done through mechanical preparation of the root canals, chemical cleaning by irrigants such as sodium hypochlorite (NaOCl) and chlorhexidine (CHX), and the placement of intracanal medication such as calcium hydroxide ($Ca(OH)_2$) paste. [6]

CHX was considering done of the most common irrigants used during endodontic treatment. [7] It has many advantages, such as antibacterial properties, biocompatibility to periapical tissues, and less cytotoxicity. [8]

Lately, herbal irrigants have been introduced in dentistry because they have several specifications, such as easy availability and low cost. Furthermore, they have antimicrobial and anti-inflammatory properties with low cytotoxicity. [9]One of the most prevalent herbal extracts used in endodontic treatment was curcumin.[10] It is a natural component extracted from turmeric plants. It has antimicrobial and antioxidant characteristics. And it also has the ability to induce the mineralization process. [11]Recently, nanotechnology has attracted a lot of attention in the last few decades because it improves the basic qualities of materials. [12]And an example of that is that nanoparticles of curcumin have evolved the valuable properties of curcumin and lessened its disadvantages. [13, 14]

Since root canal irrigants produce incomplete eradication of microorganisms, lasers were recently introduced in dentistry, hoping to get rid of all intra canal microorganisms completely. Lasers have the ability to enter deeply into dentinal tubules, unlike irrigants.[15]

Diode lasers were widely utilized because they have many advantages, such as low cost, affordability, flexibility, and small size. [16]These characteristics make it capable of permeation into curved canals and proper allocation of the laser light into the dentinal tubules. And hence, this results in the elimination of most root canal microorganisms. [17]

But complete killing of microorganisms is not achieved by irrigant alone or by laser alone. [18]So we are aiming to combine the benefits of all recent modalities such as nanotechnology, herbal irrigants, and lasers to achieve a complete sterilization of the root canal system to increase the success rate of endodontic treatment.

The purpose of our study was to compare the antibacterial efficacy of 0.125% CHX and nanocurcumin when used alone and in combination with a 980 nm diode laser against *Enterococcus faecalis* biofilm. The null hypothesis was that the combination of nanocurcumin irrigant and a 980 nm diode laser was able to eliminate *Enterococcus faecalis* completely from the root canals.

Materials and methods:

Sample size calculation:

It was calculated based on a past study by Mathew*et al.*[19] as a reference. According to this study, the minimum acceptable number of sample size was 12 per group, as the response within each subject group was normally distributed with standard deviation1.97×108. If the genuine difference was 2.3 × 108, when probability (power) was 0.8., the Type I error probability associated with this test was 0.05. The sample size was prepared using P.S Power 3.1.6. software.

Ethical Approval:

The study was approved by the Research Ethical Committee (REC) of the National Institute of Laser Enhanced Sciences on 3/1/2023, and the approval reference was: NILES-EC-CU 23/3/1.

Selection of the samples of the study:

Forty eight extracted human single rooted teeth with a mature apex were chosen. They were gathered from patients whose teeth were extracted because of orthodontic treatment. They were tested for the absence of fractures, resorption or cracks. [20]

Preparation of the teeth:

The teeth were prepared by removing their crowns at the cementoenamel junction (CEJ) by stone bur in a high speed motor. The length of the roots was standardized to 13+/-1 mm. Working length was established to be one mm shorter than the root apex. Mechanical preparation was done by M-pro rotary files according to the manufacturer's instructions.

One ml of 2.5% NaOCl was used as an irrigant between each file, and then the roots were irrigated with one ml of 17% ethylenediaminetetraacetic acid (EDTA) for the removal of the smear layer, followed by one ml of saline and then one ml of 2.5%

NaOCl. Finally, roots were irrigated with 5ml of saline.

Closure of the apices of the roots was done with light-cured composite resin to restrain apical leakage. To prevent external bacterial contamination, roots were covered by two layers of varnish. [20]

All roots were sterilized in an autoclave at 121 °C for 15 minutes. All subsequent stages were done in laminar air flaw.

Preparation of bacterial suspension and infection of the roots:

Enterococcus faecalis ATCC 29212 was obtained from the Microbiological Department, Faculty of Medicine, Cairo University. The bacterial suspension was standardized to 0.5 McFarland. Each root was placed in an Eppendorf tube and injected with 10ml of *Enterococcus feacalis* suspension with a sterile syringe. Eppendorf tubes were placed inside an incubator at 37 ºC under anaerobic conditions for 3 weeks. The bacterial suspension was renewed every 3 days to get a mature biofilm of *Enterococcus feacalis*.

Laser used in the study:

A high power 980 nm diode laser* with an output power of 2 watts through 400 um optical fibers was used in our study.

* Ezlase, Epic X, Biolase, CA, USA.

Formation of nanocurcumin:

Nanocurcumin was synthesized at Nawah Scientific Company (Cairo, Egypt) by the wet milling method [21], in which distilled water was used in this fabrication method.100 mg of curcumin was dissolved in 20 ml of dichloromethane solvent to get a solution, which was sprayed with a flow rate of 0.2 mL/min into boiling water drop wise. And it was agitated by ultrasonic's with 200 W powers and a frequency of 30 kHz for 30 minutes. After the agitation process, the solution was centrifugal 800 rpm for about 20 minutes, until an orange-colored, clear solution was collected.

Classification of the root canals:

Forty eight root canals were randomly divided into four groups according to the type of irrigant used. Each group was composed of twelve root canals. They were treated as follows:

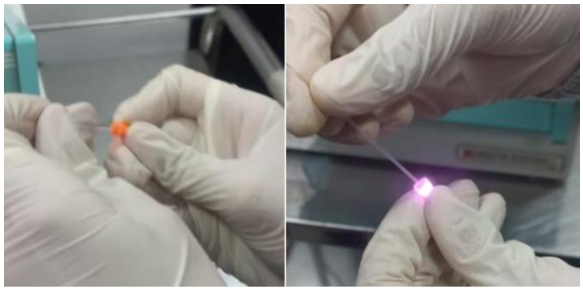
Group A: The root canals were irrigated with 2ml of normal saline for one minute. It acts as a positive control group.

Group B: The root canals were irrigated with 2 ml of 0.125% CHX for one minute.

Group C: The root canals were irrigated with 2 ml of nanocurcumin for one minute.

Group D: The root canals were irrigated with nanocurcumin irrigant, then activated

by a 980 nm diode laser with an output power of 2watts through a 400 um optical fiber used in a helical movement inside the root canals for 15 seconds, which was reduplicated for four cycles, with the total exposure time being one minute, as shown in figure (1).



Figure(1): Application of diode laser after irrigation of nanocurcumin in group (D).

Sampling procedures:

First sample (before treatment sample): Bacterial samples were collected from the infected root canals before treatment by sterile paper points.

Second sample (after treatment sample): Bacterial samples were collected from the infected root canals after treatment by sterile paper points.

Culture of the samples:

After the collection of the samples, each paper point was placed in an Eppendorf tube containing 0.5ml of brain heart infusion (BHI) broth. Fifty µl from each Eppendorf tube was cultured on bile esculin agar plates and incubated at 37 °C for 24 hours. Colony forming units (CFUs) were calculated per ml before and after treatment.

Statistical analysis:

All our data were presented as maximum, minimum, mean, and standard deviation. Data were represented in one table and two graphs. Statistical analysis was done with SPSS 16 ® (Statistical Package for Scientific Studies), Graph pad prism, and windows excel.

Inspection of the study data was completed using Shapiro-Wilk test and Kolmogorov-Smirnov test to check the normality, which revealed that the significant level (P-value) was insignificant as P-value > 0.05, which referred to the fact that the alternative hypothesis was unacceptable, and the concluded data was generated from non-parametric data. Accordingly, comparing between two different intervals was done by using Wilcoxon Rank Sign, while comparing between four different groups was done by using Kruskal Wallis test.

Results:

1-Comparison between before and after treatment in all groups:

There was a significant decrease in all groups as P<0.05 as the reduction percentage was 93.5% in CHX irrigant (group B), 90.26% in nanocurcumin irrigant

(group C), and 99.5% in nanocurcumin irrigant with a 980 nm diode laser (group D), except in the control group with saline irrigant (group A), where there was an insignificant decrease as the reduction percentage was 0.15%, as shown in table (1) and figures (2, 3).

Table (1): Comparison between before and after treatment in all groups:

Groups	Interval	N	Min	Max	M	SD	% of reduction	*P-value* Wilcoxon Rank Sign
Group A	**Before**	12.00	138.00	155.00	148.17	5.72	0.18%	0.89
	After	12.00	139.00	155.00	147.92	5.43		
Group B	**Before**	12.00	139.00	155.00	147.92	4.87	93.5%	0.002*
	After	12.00	6.00	12.00	9.58	1.62		
Group C	**Before**	12.00	138.00	154.00	148.08	5.43	90.26%	0.002*
	After	12.00	13.00	16.00	14.42	1.08		
Group D	**Before**	12.00	139.00	166.00	150.83	8.92	99.5%	0.002*
	After	12.00	0.00	3.00	0.75	1.14		

Min: minimum *Max: maximum*
M:mean *SD: standard deviation*
**Significant difference as P<0.05.* *ns: non-significant as P>0.05*

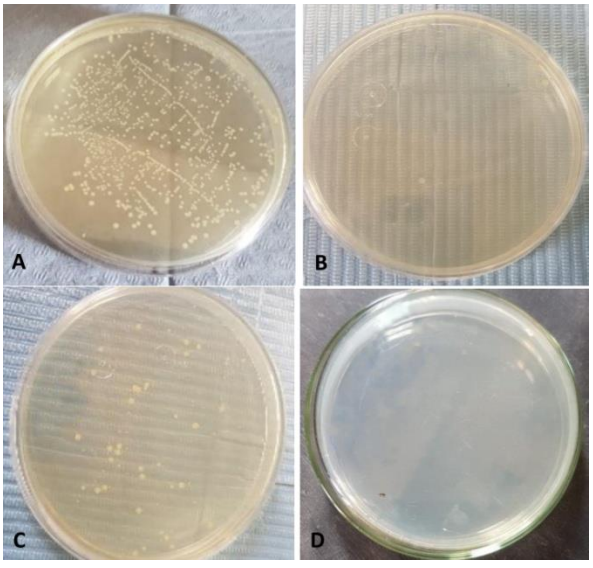


Figure (2): Agar plate cultured after irrigation with (A): saline (Group A), (B): 0.125% CHX (Group B), (C): nanocurcumin (Group C), and (D) nanocurcumin and diode laser (Group D).

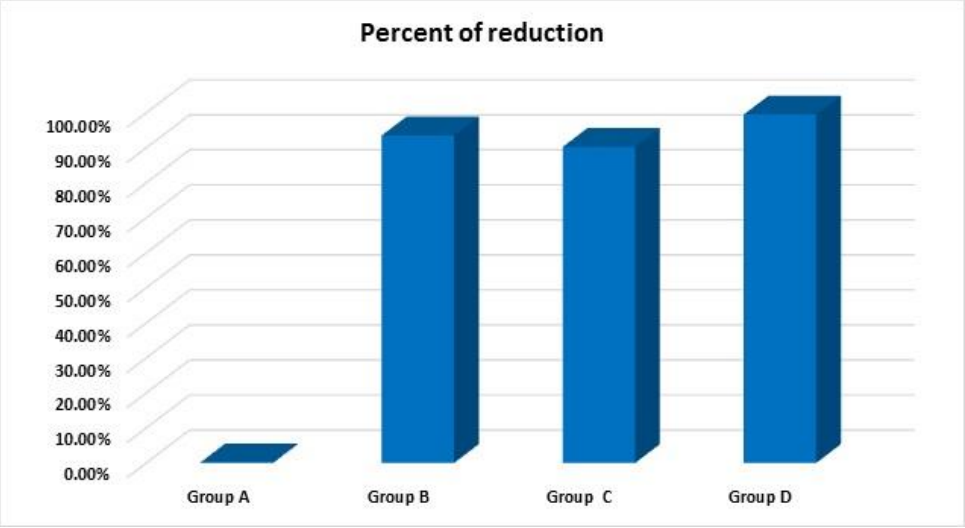


Figure (3): bar chart showing percent of reduction in all groups.

2-Comparison between different groups:

In the before treatment groups, there was an insignificant difference between all groups as P=0.85, while after treatment groups, there was a significant difference as P=0.001, as control group with saline irrigant (group A) was significantly the highest (147.92 ± 5.43), followed by thegroup of CHX irrigant (group B) (9.58 ±1.62), and the group of nanocurcumin irrigant (group C) (14.42 ±1.08), with an insignificant difference between them, while the group nanocurcumin irrigant with 980 nm diode laser (group D) (0.75 ±1.14) was significantly the lowest, as shown in table (1) and figure(4).

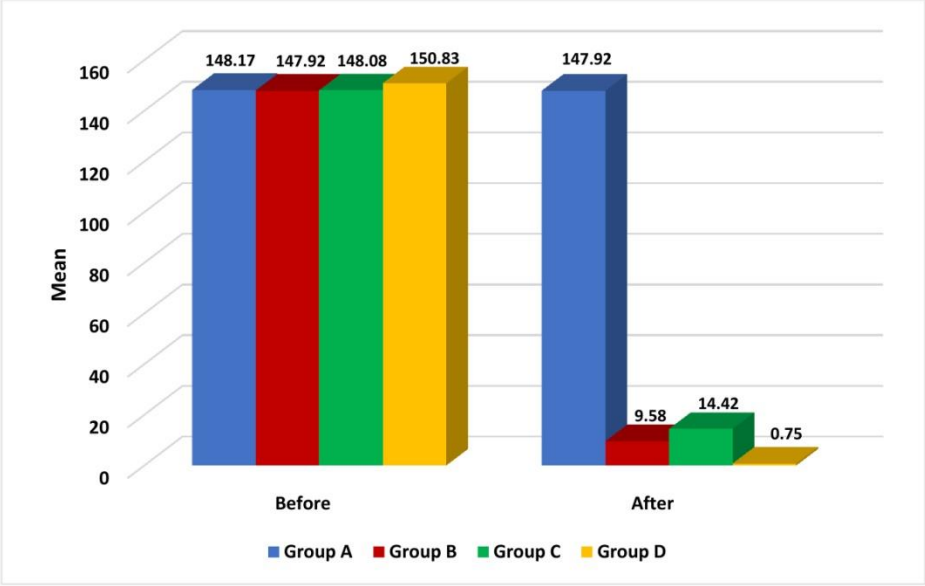


Figure (4): bar chart showing Comparison between different groups.

Discussion:

The goal of chemical cleaning in endodontic treatment is to eradicate the microorganisms completely from the root canals, but there are some difficulties. These difficulties don't lie only in finding an irrigant with superior antibacterial properties. But it also lies in the complexity of the root canals, in which microorganisms can persist and enter deeply into the dentinal tubules in areas where the irrigant can't reach them perfectly. [22]

The aim of our study was to compare the antibacterial efficacy of 0.125% CHX and nanocurcumin when used alone and in combination with a 980 nm diode laser against *Enterococcus faecalis* biofilm.

Group Awas designed to be a positive control group and irrigated with saline to assure that *Enterococcus faecalis* entered deeply into dentinal tubules, forming biofilm. *Enterococcus faecalis* was chosen as a tested microorganism in the study because it is the most commonly detected microorganism in cases with secondary infections and retreatments. [23]

The results of this study stated that the reduction percentage of numbers of *Enterococcus faecalis* after treatment with 0.125% CHX irrigant in group B was 93.5 %. This may be attributed to the solitary characteristic of CHX irrigant, which is called substantivity. It means that it has the ability to adhere to root canal dentin, which leads to prolonged liberation of its efficacy. It is also abroad spectrum antibacterial agent. [19]And this is related to its characteristic of being a cationic agent. This property enables it to bind to the anionic portion of the surface of the microorganisms (lipopolysaccharide in gram-negative bacteria and phosphate groups in gram-positive bacteria), resulting in damage to the cytoplasmic membrane of the microorganisms. [24]This result was in accordance with the results of Mathew*et al.*[19], who proved that CHX irrigant has antibacterial efficacy against *Enterococcus faecalis*. Also with the results of Duan*et al.*[25], Zhang*et al.*[26], and Lv*et al.*[27]who stated that CHX has a strongly antibacterial effect against *Enterococcus faecalis*. Furthermore, with the results of Abdullah*et al.*[28] who stated that CHX was effective even at low concentrations against root canal microorganisms. The result of this study was against the results of Vaziri*et al.*[29] who stated that CHX irrigant was not efficient in killing *Enterococcus faecalis*. This may be attributed to the use of different concentration of CHX irrigant.

While the reduction percentage of numbers of *Enterococcus faecalis* after treatment

with nanocurcumin irrigant in group C was 90.26%. This may be attributed to the antibacterial efficacy of nanocurcumin against gram-negative and gram-positive species. [30] It also has an antibacterial effect against bacterial species that have antibiotic resistance and inhibit bacterial biofilm. [31] Its mechanism of action was to destroy the cell membrane of the bacteria, prevent bacterial quorum sensing, and confuse cellular procedures by targeting bacterial DNA.[32, 33]This result was in accordance with the results of Shalan[34], who proved that curcumin irrigant could eliminate *Enterococcus faecalis* biofilms. And with the results of Abdou and Mohamed [35] and Panwar*et al.* [36] who stated that nanocurcumin hasan antibacterial effect against *Enterococcus faecalis*. While our result was in disagreement with the results of Chaitanya*et al.*[37] who stated that turmeric extracts could not eliminate *Enterococcus faecalis*. This may be related to the use of a different formulation of curcumin because they did not use it as nanocurcumin.

Furthermore, the reduction percentage of numbers of *Enterococcus faecalis* after treatment with 0.125% CHX irrigant in group B was higher than the group treated with nanocurcumin irrigant in group C. This result was in accordance with the results of Aboubakr*et al.*[38] who stated that CHX irrigant had more antibacterial efficacy than curcumin.

The highest percentage reduction in numbers of *Enterococcus faecalis* was detected in group D treated with nanocurcumin and a 980 nm diode lasers, which was 99.5%. This may be related to the combination of the antibacterial efficacy of both nanocurcumin and diode laser. The bactericidal effect of diode laser was achieved through thermal photo disruptive activity. As it interacts with the hydroxyapatite and water contents of root dentin.[39]Also, it has the capability of removing dentin debris and the smear layer, which leads to easy accessibility to complex areas of root canals and hence increases its bactericidal effect. This result was in line with the results of Polenik[40],who stated that the combination of curcumin nanoparticles and laser led to the elimination of endodontic microorganisms, especially in inaccessible areas. Also, these results were in line with those of Devaraj*et al.*[41], who concluded that the use of curcumin as a root canal irrigant with laser showed higher antibacterial efficacy against *Enterococcus fecalis* biofilm.

The limitation of our study was that it was an *in vitro* study; therefore, we recommend assessing this combination of laser and nonocurcumin irrigant *in vivo* and using long-dated clinical tests.

Conclusion:

The combination of nanocurcumin irrigant and a 980 nm diode laser is a promising modality in elimination of *Enterococcus faecalis* completely from the root canals.

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