Evaluation of the effect of 980 nm diode laser intracanal irradiation on postoperative pain and root canal disinfection in endodontic retreatment cases with chronic periapical lesions (A randomized clinical trial).

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ABSTRACT

Introduction: Endodontic retreatment cases are usually associated with postoperative pain (PP) and inflammation, due to the accumulation of bacteria inside root canals such as the E. faecalis bacteria. High-level diode laser intracanal irradiation was proposed as an effective method of root canal disinfection and decreasing PP following the primary endodontic procedures. However, there is no relevant evidence about its effect in the retreatment cases. **Aim:** To evaluate the effect of 980 nm diode laser intracanal irradiation on postoperative pain and root canal disinfection in endodontic retreatment cases with chronic periapical lesions. **Methods:** This is a self-funding randomized clinical trial. The protocol was registered at www.clinicaltrials.gov (Identifier: NCT03711357). Sixty participants with retreatment cases were selected and randomly divided into two groups (n=30); laser and placebo groups. The retreatment procedures were done over two visits, the first visit included old gutta percha removal, chemo-mechanical preparation and 980 nm diode laser intracanal irradiation for laser group. For placebo group, the laser fiberoptic tip was inserted inside root canals without activation. The second visit, 7 days later, the obturation was performed. PP was recorded at 6, 24, 48, 72 hours and 7 days postoperatively, using a modified VAS scale. To assess the intracanal E. faecalis counts, four microbiological samples were taken using sterile paper points; preoperatively, after chemo-mechanical preparation, after laser application and recolonization sample, using the bacterial culture method. The CFU/ml was calculated. The analgesic tablet consumption was evaluated as well. The significance level was set at $P \leq 0.05$. **Results:** The laser group showed less PP at the first 72 hours postoperatively compared to the placebo group, however this difference was non statistically significant. There was significant decrease in the E. faecalis counts after laser application and at the recolonization sample. Moreover, the laser group showed significantly less number of consumed analgesic tablets at the first day. **Conclusions:** 980 nm diode laser intracanal irradiation can decrease postoperative pain and minimize the analgesic tablet consumption following the endodontic retreatment procedures. Moreover, it can better eliminate the E. faecalis bacteria from the infected root canals and decrease the bacterial recolonization.

Keywords: Diode, laser, pain, endodontic, retreatment, disinfection.

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INTRODUCTION

Endodontic retreatment cases are very challenging cases to deal with. They have significantly higher incidence of postoperative pain (PP) and flare ups (Gorni and Gagliani, 2004), which definitely leads to patients' dissatisfaction and affects negatively their quality of life (Vena *et al.*, 2014; Pasqualini *et al.*, 2016). Postoperative pain is due to inflammation (Siqueira *et al.*, 1997), which is mainly due to the accumulation of different types of aggressive and resistant bacteria in the infected root canals such as Enterococcus faecalis (E.faecalis) (Saydjari, Kuypers and Gutknecht, 2016).

Conventional techniques of root canals disinfection such as mechanical instrumentation, irrigating solutions and intracanal medicaments are not always sufficient to render root canals free of bacteria (Siqueira *et al.*, 1997). The anatomical complexities, the shallow penetration of irrigating solutions inside the dentinal tubules (not more than 100 µm with the conventional syringe irrigation method) as well as the bacterial growth as biofilms render complete eradication of microorganisms from the root canal system almost impossible (Mohammadi *et al.*, 2013).

Several techniques have been introduced in order to better disinfect root canals, in an attempt to reduce pain and inflammation associated with endodontic procedures (Garcez *et al.*, 2007; Siqueira and Rôças, 2008).

High power laser intracanal irradiation (such as diode laser) has been introduced as a new approach of root canal disinfection (Saydjari, Kuypers and Gutknecht, 2016). In combination with the conventional techniques, they can overcome the limitations to disinfect root canals (Yoo *et al.*, 2014). They allow the access to the unavailable areas of root canals, such as the tubular network (Preethee *et al.*, 2012). They have a penetrating power up to 1000 µm in dentin, surpassing the effective range of other chemical agents such as NaOCl (Pirnat, 2007). They could achieve a bacterial reduction of 98.8% (Beer *et al.*, 2012),and have shown good bactericidal action on E. feacalis bacteria even in deeper layers of dentin (Mortiz *et al.*, 1997; Gutknecht *et al.*, 2002; Schoop *et al.*, 2004).

A research question has arisen, "In endodontic retreatment cases with chronic periapical lesions, does the use of 980 nm diode laser intracanal irradiation versus placebo, affect postoperative pain and root canal disinfection?". The aim of the study was to determine the effect of 980 nm diode laser on Postoperative pain, root canal disinfection and number of analgesic tablet consumption, in endodontic retreatment cases with chronic periapical lesions.

To the best of our knowledge, there is no available relevant research about the effect of 980 nm diode laser intracanal irradiation on

postoperative pain in endodontic retreatment cases.

SUBJECTS AND METHODS

Study design & registration:

This randomized clinical trial has been written according to the Preferred Reporting Items for Randomized Trials in Endodontics (PRIRATE) 2020 guidelines (Nagendrababu et al., 2020). It was designed as a randomized placebo-controlled clinical trial. The superiority framework trial with two parallel groups randomization was performed as simple randomization with 1:1 allocation ratio. The study protocol was registered at www.clinicaltrials.gov (ClinicalTrials.gov Identifier: NCT03711357).

Ethical considerations:

The protocol of the trial was approved by the institutional review board/ethical committee in the Faculty of Dentistry (Approval number: 181018). The treatment procedures, aim of the study, possible side effects, and treatment alternatives were thoroughly explained for all the participants. Participants were asked to sign a printed informed consent.

Study settings:

Recruitment, treatment and follow-up of the study participants were done by the main investigator, who is a Ph.D. candidate of endodontics, faculty of dentistry. All the participants were recruited from the clinic of the endodontic department, Faculty of Dentistry, in the duration between October 2018 – October 2022. All the eligible participants were enrolled.

The participants were treated according to the declaration of Helsinki and as revised in 2013. All patients signed an informed consent after a comprehensive explanation of the treatment procedures, the side effects and the treatment alternatives.

Inclusion and exclusion criteria:

Inclusion criteria:

All the selected cases were endodontic retreatment cases of single rooted teeth with chronic periapical lesions; with small sized periapical radiolucencies (≤ 5 mm in diameter). The age of subjects ranged between 18-50 years of age, with no contributory medical history, and with no previous administration of analgesics within the previous 12 hours and / or antibiotics within the previous month.

Exclusion criteria:

Retreatment cases with complications (e.g. curved root canals, separated instruments, perforations, ledges, resorption, transportation, calcifications, immature apices and over fillings), swelling or sinus tracts were excluded. Similarly, mutilated teeth with difficulty of isolation, pregnant or nursing females were excluded as well.

Sample size calculation:

The power analysis used pain (VAS) scores as the primary outcome. Based upon the results of (Yoo *et al.*, 2014), the effect size was found to be 0.8075. Using alpha (α) level of (5%) and Beta (β) level of (20%) i.e. power = 80%; the minimum estimated sample size was 27 subjects per group for a total of 54 subjects. To compensate for a drop-out rate of

about 15%, the number was increased to be a minimum of 30 subjects per group for a total of 60 subjects. Sample size calculation was performed using G*Power Version 3.1.9.2.

Randomization:

Random sequence generation (Randomization): was generated using random sequence generator internet site (https://www.random.org/sequences/), on 2 groups. One of the co-authors named both groups; Laser group and Placebo group, and kept the tables of the random sequence with him.

Allocation concealment mechanism: Each number was written in a separate eight folded piece of paper, then each paper was placed in an opaque sealed envelope.

Implementation:

After completing the chemo-mechanical preparation procedures, the envelopes were shuffled, and each subject drew an envelope. The envelope was opened by the operator, to uncover the number written on the paper. A telephone call was made with the co-author, to determine which group the subject belongs to, according to tables of random sequence.

Blinding (Masking):

This study was a "single-blind" study; as all the subjects did not know which interventional group they belong to.

Interventions:

First visit:

Selection of cases: Sixty subjects with endodontic retreatment cases of single rooted teeth with chronic periapical lesions were selected. The size of the periapical lesions was determined using the digital radiography in a paralleling technique, by measuring the maximum horizontal dimension of each lesion, representing its diameter. All the eligible cases were recruited in the study.

Assessment of preoperative pain: was recorded using "modified visual analogue scale" (VAS scale). All the recruited cases were chronic asymptomatic cases, with no preoperative pain.

Retreatment procedures: Under rubber dam isolation, the gutta percha in the coronal two thirds of the root canals was removed using gates glidden drills (#3 and #2), and in the apical one third using H- files and gutta percha solvent, and a digital radiograph was taken to confirm the removal of the old filling material. Working length was determined using an electronic apex locator, and then was confirmed radiographically.

1st microbiological sample (Initial sample): Each canal was irrigated using 1ml of sterile saline solution. The canals were dried using 3 sterile paper points, of a size equivalent to the size of the largest H-file used for gutta percha removal, with 2% taper. The paper points were placed up to the full working length, and left for 1 minute each. The paper points were removed from the root canal, and placed in a sterile glass tube containing "Thioglycollate" solution as a transport medium.

Chemo-mechanical preparation: All root canals were prepared using ProTaper Universal rotary file system, in conjunction with a 1:16 gear reduction handpiece, powered by an X-Smart endodontic motor. All root canals were prepared up to the full working length, to a size not less than #30 (F3 file), to allow the optical fiber to reach the apical part of root canal. If root canals were to be enlarged to files larger than F3 files, F4 and F5 ProTaper Universal files were used. And for larger canals, K -and H-manual files were used.

Irrigation: 2 ml of 5.25% NaOCl solution, was done between each two successive files, with a total irrigation time of 40 min. for each canal. This was followed by a flush of 17% ethylene diamine tetra-acetic acid (EDTA) solution (1 ml of EDTA soln. for 1 min. for each canal). A flush of 3 ml of saline solution was used between both irrigants. Final irrigation was made using 5 ml of sterile normal saline solution. All the irrigation steps were done using a 30- gauge needle.

2nd microbiological sample (After chemo-mechanical preparation): The canals were dried using another 3 sterile paper points, with a size equivalent to the size of the master apical file with 2% taper, each left in root canal for 1 minute, and were stored as before.

Laser application (Laser group): The canals were irradiated using a high power diode laser device, with the following parameters; 980 nm wavelength, 3 watts output power, pulsed mode, 10.00 Hz frequency, 100 ms pulse time and coupled with a fiber optic tip of 200 µm diameter. The length of the fiberoptic tip was adjusted to be 1mm shorter than the working length, inserted inside the root canal and then was removed in a spiral motion from apical to coronal towards the orifice (Fig. 1). Four irradiations were performed, of 5 seconds each, with a rest period of 5 seconds between each two successive irradiations.

Mock laser application (Placebo group): The fiberoptic tip was inserted inside the dry root canal mimicking the laser irradiation, but without activation.

A 3rd microbiological sample was taken (After laser application). A piece of dry cotton was placed in the access cavity, and then was sealed with glass ionomer filling material, to prevent leakage.

Assessment of Postoperative pain levels:

At the end of the first visit, the participants were given the (modified VAS) scale charts, and were instructed to record their postoperative pain levels at 6, 24, 48, and 72 hours, and 7 days after root canal treatment. Criteria to determine the level of postoperative pain were as follows; 0: no pain, 1–3: mild pain, 4–6: moderate pain and 7–10: severe pain.

Assessment of "number of analgesic tablet consumption":

The participants were given a rescue bag of 12 analgesic tablets Ibuprofen 600 mg (Brufen 600 mg tablets, Abbott Pharmaceuticals, Italy), and instructions in case of pain, he/she could could use the analgesic with a maximum dose of one tablet every 6 hours. In addition, they

were asked to record the number of analgesic tablets used, at day 1, day 2, day 3 and day 7 postoperatively, in the "number of analgesic tablet consumption" chart.

The participants were asked to bring back their "modified VAS" charts and the "number of the analgesic tablet consumption" charts at the 2nd visit.

Second visit (7 days later):

All the recruited participants presented to the 2nd visit, without any drop outs.

The teeth were isolated, the glass ionomer filling material was removed, the root canals were irrigated using 5 ml of sterile saline solution and a 4th microbiological sample was obtained, to assess *the bacterial recolonization*.

Obturation was carried out using a "modified single cone technique", using 4% taper gutta-percha master cones & 2% taper gutta percha auxiliary cones, and resin-based sealer. The access cavity was then sealed using a temporary restorative material, and the subjects were referred to the operative department for placement of the final restoration.

Microbiological analysis:

All the microbiological samples were immediately sent to the microbiology laboratory at the Department of Microbiology, Faculty of medicine. Once the paper point samples arrived at the microbiology laboratory, Faculty of Medicine, they were removed from the "Thioglycollate" transport medium, and diluted using 1.5 ml Brain Heart Infusion (BHI) broth, and 0.6% Sodium Thiosulfate (to prevent continued antimicrobial effects of the sodium hypochlorite irrigant on samples) (Vajrabhaya *et al.*, 2017). The samples were then vortexed in a microcentrifuge for 30 sec. 100 µl aliquots were added to wells of a 96 well plate for serial dilution. Diluted samples were placed on Brain Heart Infusion (BHI) Agar plates, in order to be cultured. The samples were then incubated at 37°C, for 48 hours in case of anaerobic jars of the ***facultative anaerobic E. Faecalis bacteria***. After incubation, the colonies of the *E. faecalis* bacteria were counted. The result was multiplied by the reversed dilution coefficient, and the result were reported as Colony forming unit per ml (CFU /ml) (Fig. 2,3).

Statistical methods:

Qualitative data were presented as frequencies and percentages. Chi-square test and Fisher's Exact test were used to compare between the two groups. Numerical data were explored for normality by checking the distribution of data and using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). Logarithmic transformation of bacterial counts data was performed due to the high range of bacterial counts.

All data showed non-normal (non-parametric) distribution except for age data which showed normal distribution. Data were presented as median, range, mean and standard deviation (SD) values.

For parametric data, Student's t-test was used to compare between the two groups. For non-parametric data, Mann-Whitney U test was

used to compare between the two groups. Friedman's test was used to study the changes within each group. Dunn's test was used for pair-wise comparisons when Friedman's test is significant.

The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

RESULTS

1. The demographic data:

The demographic data; age and gender showed no statistically significant difference between both groups (P= 0.632 and 1 respectively) (Table 1).

2. Post-operative pain:

a. Changes within each group:

As regards Laser group; there was a ***statistically significant change in the prevalence of pain by time*** (*P*-value <0.001, Effect size = 0.351). At 6 hours, there was an increase in prevalence of "mild" pain and a decrease in prevalence of "no pain". Then from 6 to 24 hours; an increase in prevalence of "no pain" was observed associated with a decrease in prevalence of "mild" pain. From 24 to 48 hours, 48 to 72 hours as well as 72 hours to 7 days, no change in prevalence of pain was observed.

As regards Placebo group; there was a ***statistically significant change in the prevalence of pain by time*** (*P*-value <0.001, Effect size = 0.27). At 6 hours, there was an increase in prevalence of "mild", "moderate" pain and a decrease in prevalence of "no pain". From 6 to 24 hours; there was an increase in prevalence of "no pain", a decrease in prevalence of "mild" pain while prevalence of "moderate" pain didn't change. From 24 to 48 hours; there was an increase in prevalence of "no pain" and a decrease in prevalence of "moderate" pain while prevalence of "mild" pain didn't change. From 48 to 72 hours as well as 72 hours to 7 days; there was an increase in prevalence of "no pain" and a decrease in prevalence of "mild" pain while there were no cases with moderate pain along these three follow up times (Fig.4) (Table 2).

b. Comparison between both groups:

Pre-operatively; all cases in both groups had no pain, so no statistical comparison could be performed.

At 6, 24, 48, 72 hours as well as seven days postoperatively; there was ***no statistically significant difference between the prevalence of pain between both groups*** (*P*-value = 0.318, Effect size = 0.231), (*P*-value = 0.227, Effect size = 0.246), (*P*-value = 1, Effect size = 0.076), (*P*-value = 1, Effect size = 0) and (*P*-value = 1, Effect size = 0.13), respectively (Table 3).

1. Intracanal E. faecalis counts (CFU/ml):

a. Changes within each group:

As regards Laser group; there was a ***statistically significant*** decrease in the median Log_{10} CFU/ml of E.faecalis counts

(*P*-value <0.001, Effect size = 0.802). Pair-wise comparisons revealed that there was a ***statistically significant*** decrease in Log_{10} CFU/ml of E.faecalis counts after chemo-mechanical preparation as well as after laser application. After re-colonization; the median Log_{10} CFU/ml of E.faecalis counts showed non-statistically significant difference from the values after Laser application but a statistically significant lower value compared with pre-operative counts as well as counts after chemo-mechanical preparation (Fig. 5) (Table 4).

As regards Placebo group; there was a ***statistically significant*** decrease in median Log_{10} CFU/ml of E.faecalis counts (*P*-value <0.001, Effect size = 1). Pair-wise comparisons revealed that there was a ***statistically significant*** decrease in Log_{10} CFU/ml of E.faecalis counts after chemo-mechanical preparation. After re-colonization; the median Log_{10} CFU/ml of E.faecalis counts showed non-statistically significant difference from the values chemo-mechanical preparation but a statistically significantly lower value compared with pre-operative counts (Fig.6) (Table 4).

b. Comparison between both groups:

Pre-operatively as well as after chemo-mechanical preparation; there was no statistically significant difference between median Log_{10} CFU/ml of E.faecalis counts in both groups (*P*-value = 0.533, Effect size = 0.159) and (*P*-value = 0.156, Effect size = 0.371).

After re-colonization; Laser group showed ***statistically significantly*** lower median Log_{10} CFU/ml of E.faecalis counts than Placebo group (Table 5).

c. Percentage reduction in E.faecalis counts (%):

Percentage reduction was calculated as follows:

[(Pre-operative counts – Post-operative counts)/ Pre-operative counts × 100]

After chemo-mechanical preparation; there was no statistically significant difference between percentage reduction in E.faecalis counts in both groups (*P*-value = 0.263, Effect size = 0.291).

After re-colonization; Laser group showed ***statistically significantly*** higher median percentage reduction in E.faecalis counts than Placebo group (*P*-value <0.001, Effect size = 2.727) (Fig. 7) (Table. 6).

2. Number of analgesic tablets consumption:

At day one; Laser group showed ***statistically significantly*** lower median number of consumed analgesic tablets than Placebo group (*P*-value = 0.011, Effect size = 0.349). After two days, three days as well as seven days; none of the cases in the two groups needed analgesics.

DISCUSSION

The selected cases for the current study were endodontic retreatment cases with chronic periapical lesions, as these cases were more susceptible to experience acute pain and flare ups following the endodontic procedures (Arslan *et al.*, 2017), with lack of evidence on the effect of diode laser on postoperative pain.

Postoperative pain is influenced by several confounding factors. A major confounding factor is the presence of preoperative pain, that was proven to significantly affect the level of postoperative pain (Nagendrababu and Gutmann, 2017). Therefore, the selected cases for the current study were chronic cases with no history of preoperative pain, to allow for better correlation of the results to the experimental groups.

Another confounding factor is the size of the periapical lesion. According to a systematic review by (Sathorn, Parashos and Messer, 2008) that the chronic periapical lesions of more than 5 mm in diameter were associated with more PP, due to higher number of bacterial strains inside the root canals, and more inflammatory reaction being induced. Therefore, small sized periapical lesions (≤5 mm in diameter) were selected.

Similarly, all the participants included, had no history of taking analgesics 12 hours prior to the intervention, as NSAIDs or oral steroidal anti-inflammatory drugs may reduce PP by blocking the COX pathway, thereby blocking the pain sensation even before its onset (Menke *et al.*, 2000; Arslan, Topcuoglo and Aladag, 2011). Also, the participants had no history of antibiotic therapy during the last month as this could have affected the microbiological results and cause disruption of the microbial flora (Glennon *et al.*, 2004).

Among the various laser types, diode laser devices have rapidly found their way into laser-assisted endodontics thanks to their small size, extreme compactness, affordability, ease of application due to fiber delivery, simple setting-up and versatility (Maturo, Perugia and Docimo, 2013). More importantly, it has proven a ***prominent bactericidal effect*** inside root canals, ***high penetration depth inside the dentinal tubules*** (up to >1000 µm) [65], ***selective absorption by microorganisms*** without heating the entire target tissue (Maturo, Perugia and Docimo, 2013).

The 980 nm diode laser in an output power of 3 Watt, has been selected for the current study, as it has shown the best bactericidal effect with the highest percentage of bacterial reduction, compared to shorter wavelengths (Kanumuru and Subbaiah, 2014) or lower power outputs (Kaiwar *et al.*, 2013). Four successive cycles of irradiation were performed, in accordance to a study by (Prażmo *et al.*, 2017) who found that repeating the irradiation cycles improves the antibacterial effect of the intracanal laser irradiation.

To minimize the thermal hazards of laser on dentin and periodontium, the laser was used in a pulsed mode rather than a continuous mode (Alfredo *et al.*, 2008), with a rest period of 5 seconds between each two successive cycles, in order to avoid the temperature rise on the external root surface and the damage to dentin and periodontium (Bergmans *et al.*, 2006).

The spiral motion of the fiberoptic tip from apical to coronal was proposed by (Gutknecht, 2008a), in order to reduce the heating of dentin, as the temperature rapidly decreases as the tip is dragged in the coronal direction. In addition, this motion ensures equal diffusion of light over the dentin

surface, therefore improving its bactericidal effect (Godbole *et al.*, 2017).

The modified visual analogue scale (Modified VAS scale) was used to evaluate the postoperative pain following several previous studies (Topçuoğlu, Topçuoğlu, and Arslan 2018; Vera et al. 2018). It is easily understandable by the patients and provides reliable, clear and valid results (Parirokh *et al.*, 2014). PP generally occurs during the first 2–3 days following the root canal treatment, with the peak of PP at 6 hours postoperatively (Genc Sen and Kaya, 2019). The pain then decreases over time, with the patients declaring no pain after 7 days (Morsy *et al.*, 2019).

On the other hand, the bacterial culture method was used to evaluate the bacterial load inside the root canals following (Morsy *et al.*, 2019). The culture method has the ability to detect the viable bacterial cells, and a strong correlation was proved between the negative bacterial cultures and a more favorable treatment outcome (Sjögren *et al.*, 1997; Waltimo *et al.*, 2005; Vianna *et al.*, 2007). It is worth mentioning that there are more advanced techniques including the flowcytometry, polymerase chain reaction (PCR) and others. However, (Estrela *et al.*, 2008) compared the results obtained from PCR and culture technique, and despite the higher sensitivity of the PCR technique, there was no significant difference between both techniques in the evaluation of the intracanal microorganisms.

Regarding the results; there was no statistically significant difference in PP between both groups at all time intervals, this was in accordance to the results of (Kaplan, Sezgin and Kaplan, 2021)**.** However, this contradicts with the results reported by (Morsy *et al.*, 2019) who reported lower levels of PP in laser group. This could be attributed to the fact that the former study was carried out on teeth with larger periapical lesions (ranging from 4-8 mm in diameter), where larger periapical lesions are usually associated with more PP (Nagendrababu and Gutmann, 2017). Another difference in the methodology could be related to the disinfection protocol, which used a lower concentration of NaOCl soln. (2.5%). It was reported in a study by (Farzaneh *et al.*, 2017) that 2.5% NaOCl was associated with more PP compared to 5.25% NaOCl, due to its lower disinfection ability**.**

On comparing the changes in PP within each group, there was a statistically significant change in the prevalence of pain by time at each group. In the laser group, at 6h, the pain tends to be *"mild"*, and then decreases towards *"no pain"* up to 7days postoperatively. On the other hand, in the placebo group, at 6 and 24h, the pain tends to range from *"mild"* to *"moderate"*, then decreases to be *"mild"* over 48-72 h, then decreases to reach *"no pain"* at 7 days postoperatively. Therefore, and despite the non-significant difference in comparison between both groups, the PP levels in the laser group seem to be lower than those of the placebo group at different time intervals.

The exact mechanism by which the diode lasers may reduce PP is still unknown. However, several studies have emphasized that the diode laser has an anti-inflammatory effect, by decreasing the production of pro-

inflammatory mediators such as; prostaglandin E2 (PGE2), interleukin 1β (IL 1β) and Tumor necrosis factor- alpha (TNF-α) and increases the production of the inhibitory prostaglandins such as PGI2 (Pawar *et al.*, 2014; Metin, Tatli and Evlice, 2018). In addition, it increases the threshold of pain by inhibiting the synthesis of the neurotransmitters such as substance P, and increasing the production of beta- endorphin and encephalin that are responsible for the relief of pain (Pawar *et al.*, 2014). It improves the immune response by increasing the level of immunoglobulins and lymphokines and lymphatic drainage (Metin, Tatli and Evlice, 2018). Moreover, an interesting finding by (Morsy *et al.*, 2019) who confirmed a strong correlation between the low levels of PP after diode laser irradiation and its strong bactericidal effect, in which microorganisms represent the main cause for inducing an inflammatory reaction in the periapical area.

Regarding the results of the root canal disinfection (intracanal counts of E.faecalis), there was a significantly higher values of bacterial reduction in the laser group that has reached up to 95.5%, compared to 40% for the placebo group. These results were coinciding with the results reported by (Romeo *et al.*, 2015) and (Morsy *et al.*, 2019). However, that was contradicting with (Sohrabi *et al.*, 2016) who reported a better antibacterial effect of NaOCl compared to the 980 nm diode laser. This could be attributed to the fact that the former study has studied the pure antibacterial effect of diode laser not in conjunction with chemical means of disinfection.

Therefore, it is clear that laser application has a prominent bactericidal effect, due to its photothermal effect on the bacterial cell wall (Preethee *et al.*, 2012). The direct heat causes a permanent destruction of the cell membrane, leading to bacterial cell death (Ahmeduddin *et al.*, 2012). It also, exerts a photo-disruptive effect on the unreachable bacteria; leading to a sublethal damage of the bacterial cell. Destruction of cell wall integrity and accumulation of denatured proteins takes place, causing the inhibition of the bacterial cell growth and successive cell lysis (Preethee *et al.*, 2012; Bhatia and Kohli, 2013). More importantly, the diode laser has a high penetration power inside the dentinal tubules; reaching up to >1000 μm (Gutknecht, 2008a). A possible explanation for this kind of light propagation; is the ability of enamel prisms and dentinal tubules to act as an optical fiber and thus allowing the diode laser to be effective in deep layers of dentin Odor *et al.*, 1996).

The current study had the advantage of being a randomized clinical trial with a relatively large sample size. However, it was restricted to cases single rooted teeth, with asymptomatic apical periodontitis, where the follow up period was considerably short. Therefore, it is recommended that further investigations are to be done in order to study the effect of the 980 nm diode laser intracanal irradiation on postoperative pain and root canal disinfection in cases with symptomatic apical periodontitis, cases with persistent root canal infection. Moreover, to study the effect of 980 nm diode laser on the healing rate and the survival rate of the endodontically treated

teeth following the various endodontic procedures, as these points were lacking in literature as well.

CONCLUSIONS

In review of the results and under the limitations of this study, it was concluded that:

- The 980 nm diode intracanal laser irradiation may lead to less postoperative pain at the first 72 hours compared to the conventional methods of cleaning and shaping alone. However, the difference between both groups was non-statistically significant.
- The 980 nm diode laser intracanal irradiation can better eliminate the E. faecalis bacteria form infected root canals and decrease the bacterial recolonization.
- The 980 nm diode laser intracanal irradiation provides the advantage of less analgesic tablet consumption at the first day following the endodontic retreatment procedures.

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Patient declaration of consent statement:

We have obtained all appropriate patient consent forms. The treatment procedures, aim of the study, possible side effects, and treatment alternatives were thoroughly explained for all the participants. Participants were asked to sign a printed informed consent.

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Tables

Table (1): Mean, standard deviation (SD), frequencies (n), percentages and results of Student's t-test and Chi-square test for comparison between base line characteristics in the two groups:

	Laser (n = 30)	Placebo (n = 30)	*P*-value
Age (Years) Mean (SD)	32.6 (9.8)	33.9 (11.5)	0.632
Gender [n (%)] Male	9 (30%)	9 (30%)	1
Female	21 (70%)	21 (70%)	

**: Significant at P ≤ 0.05*

Table (2): Descriptive statistics and results of Friedman's test for comparison between prevalence of pain at different follow up times within each group:

Time	Laser (n = 30)		Placebo (n = 30)	
	n	%	n	%
Pre-operative				
No pain	30	100	30	100
Mild pain	0	0	0	0
Moderate pain	0	0	0	0
6 hours				
No pain	18	60	17	56.7
Mild pain	12	40	10	33.3
Moderate pain	0	0	3	10
24 hours				
No pain	29	96.7	25	83.3
Mild pain	1	3.3	2	6.7
Moderate pain	0	0	3	10
48 hours				
No pain	29	96.7	28	93.3
Mild pain	1	3.3	2	6.7
Moderate pain	0	0	0	0
72 hours				
No pain	29	96.7	29	96.7
Mild pain	1	3.3	1	3.3
Moderate pain	0	0	0	0
7 days				

No pain	29	96.7	30	100
Mild pain	1	3.3	0	0
Moderate pain	0	0	0	0
P-value	<0.001*		<0.001*	
Effect size (w)	0.351		0.27	

**: Significant at P ≤ 0.05*

Table (3): Descriptive statistics and results of Fisher's Exact test for comparison between prevalence of pain in both groups:

Time	Laser (n = 30)		Placebo (n = 30)		*P*-value	*Effect size (v)*
	n	%	n	%		
Pre-operative					Not computed	
No pain	30	100	30	100		
Mild pain	0	0	0	0		
Moderate pain	0	0	0	0		
6 hours						
No pain	18	60	17	56.7		
Mild pain	12	40	10	33.3	0.318	0.231
Moderate pain	0	0	3	10		
24 hours						
No pain	29	96.7	25	83.3		
Mild pain	1	3.3	2	6.7	0.227	0.246
Moderate pain	0	0	3	10		
48 hours						
No pain	29	96.7	28	93.3		
Mild pain	1	3.3	2	6.7	1	0.076
Moderate pain	0	0	0	0		
72 hours						
No pain	29	96.7	29	96.7		
Mild pain	1	3.3	1	3.3	1	0
Moderate pain	0	0	0	0		
7 days						

No pain	29	96.7	30	100	1	0.13
Mild pain	1	3.3	0	0		
Moderate pain	0	0	0	0		

**: Significant at P ≤ 0.05*

Table (4): Descriptive statistics and results of Friedman's test for the changes in Log_{10} CFU/ml of E. faecalis counts within each group:

Time	Laser (n = 30)		Placebo (n = 30)	
	Median (Range)	Mean (SD)	Median (Range)	Mean (SD)
Pre-operative	6.41 (5.48-6.54) ^{A}	6.31 (0.28)	6.4 (5-6.7) ^{A}	6.2 (0.45)
After chemo-mechanical preparation	6 (0-6.3) ^{B}	4.78 (2.46)	5.7 (4-6.4) ^{B}	5.59 (0.58)
After Laser application	5.3 (0-6.04) ^{C}	4.02 (2.48)	-	-
After re-colonization	5 (0-6.08) ^{C}	3.09 (2.76)	6 (4.7-6.48) ^{B}	5.9 (0.48)
P-value	<0.001*		<0.001*	
Effect size (w)	0.802		1	

**: Significant at P ≤ 0.05, Different superscripts in the same column indicate statistically significant change.*

Table (5): Descriptive statistics and results of Mann-Whitney U test for comparison between Log_{10} CFU/ml of E.faecalis counts in both groups:

Time	Laser (n = 30)		Placebo (n = 30)		*P*-value	*Effect size (d)*
	Median (Range)	Mean (SD)	Median (Range)	Mean (SD)		
Pre-operative	6.41 (5.48-6.54)	6.31 (0.28)	6.4 (5-6.7)	6.2 (0.45)	0.533	0.159
After chemo-mechanical preparation	6 (0-6.3)	4.78 (2.46)	5.7 (4-6.4)	5.59 (0.58)	0.156	0.371
After Laser application	5.3 (0-6.04)	4.02 (2.48)	-	-	-	-
After re-colonization	5 (0-6.08)	3.09 (2.76)	6 (4.7-6.48)	5.9 (0.48)	<0.001 *	1.659

**: Significant at P ≤ 0.05*

Table (6): Descriptive statistics and results of Mann-Whitney U test for comparison between percentage reduction in E.faecalis counts (%) in both groups:

Time	Laser (n = 30)		Placebo (n = 30)		*P*-value	*Effect size (d)*
	Median (Range)	Mean (SD)	Median (Range)	Mean (SD)		
After chemo-mechanical preparation	58.8 (33.3-100)	63.9 (23.8)	68.3 (38.5-99.6)	68.3 (16.3)	0.263	0.291
After re-colonization	95.5 (60-100)	92 (10.9)	40 (14.3-92.5)	44.1 (21.2)	<0.001*	2.727

**: Significant at P ≤ 0.05*

Figures

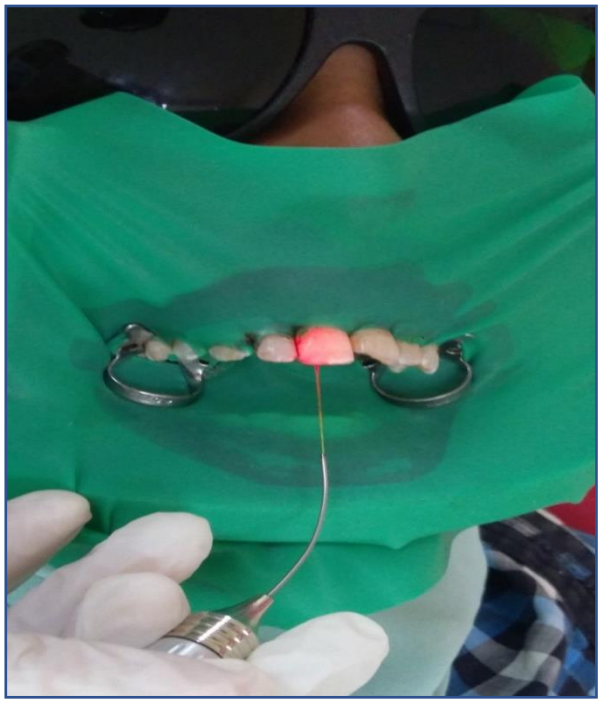


Fig. (1): The intracanal diode laser irradiation.

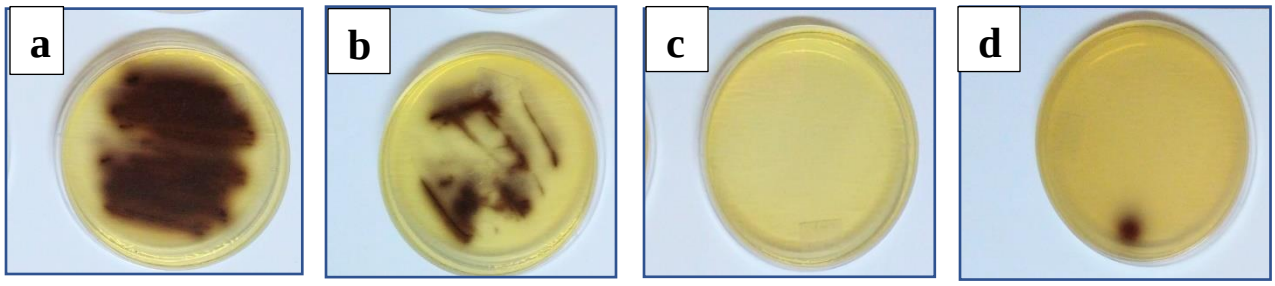
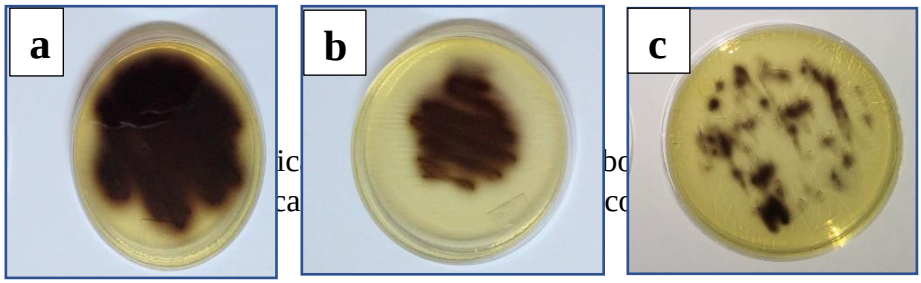


Fig. (2): The microbiological samples for the diode laser group: **(a):** initial, **(b):** after chemo-mechanical preparation, **(c):** after laser application and **(d):** recolonization samples.



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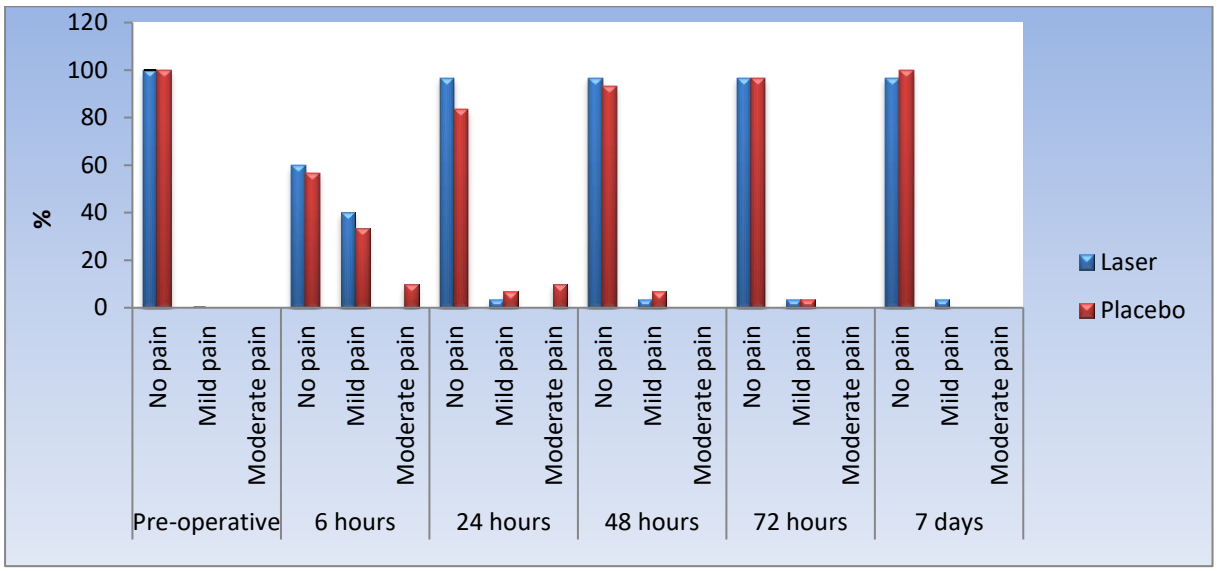

Fig. (4): Bar chart representing prevalence of pain in the two groups

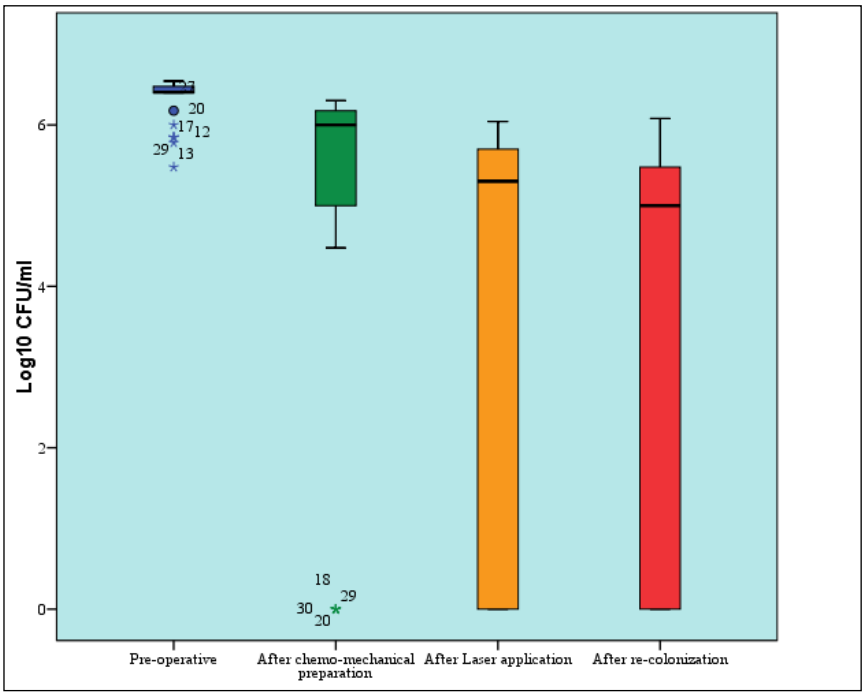

Fig. (5): Box plot representing median and range values for Log_{10} CFU/ml of E.faecalis counts in Laser group (Circle and stars represent outliers).

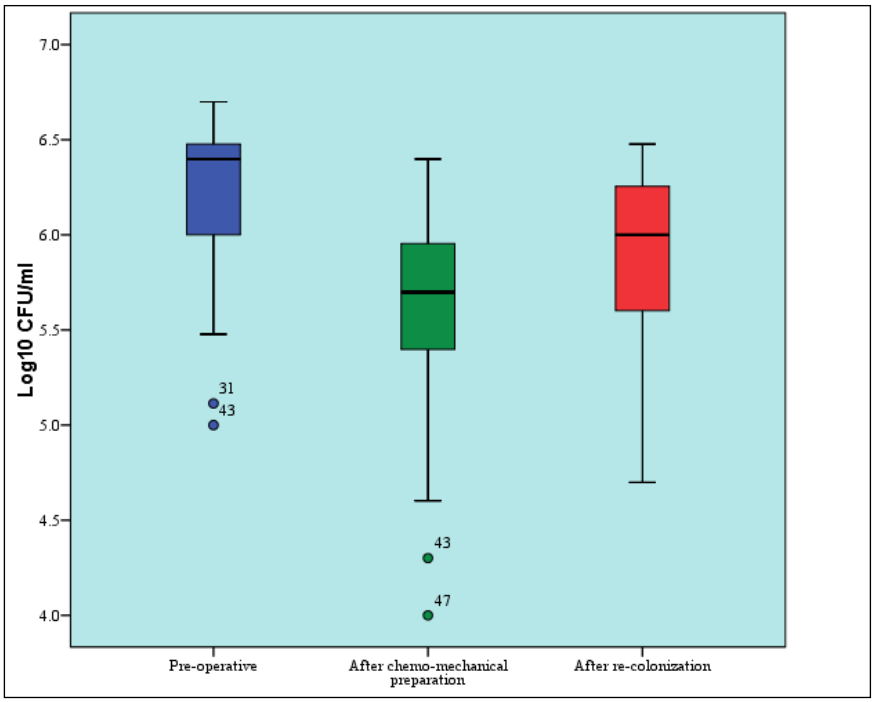

Fig. (6): Box plot representing median and range values for Log_{10} CFU/ml of E.faecalis counts in Placebo group (Circles represent outliers).

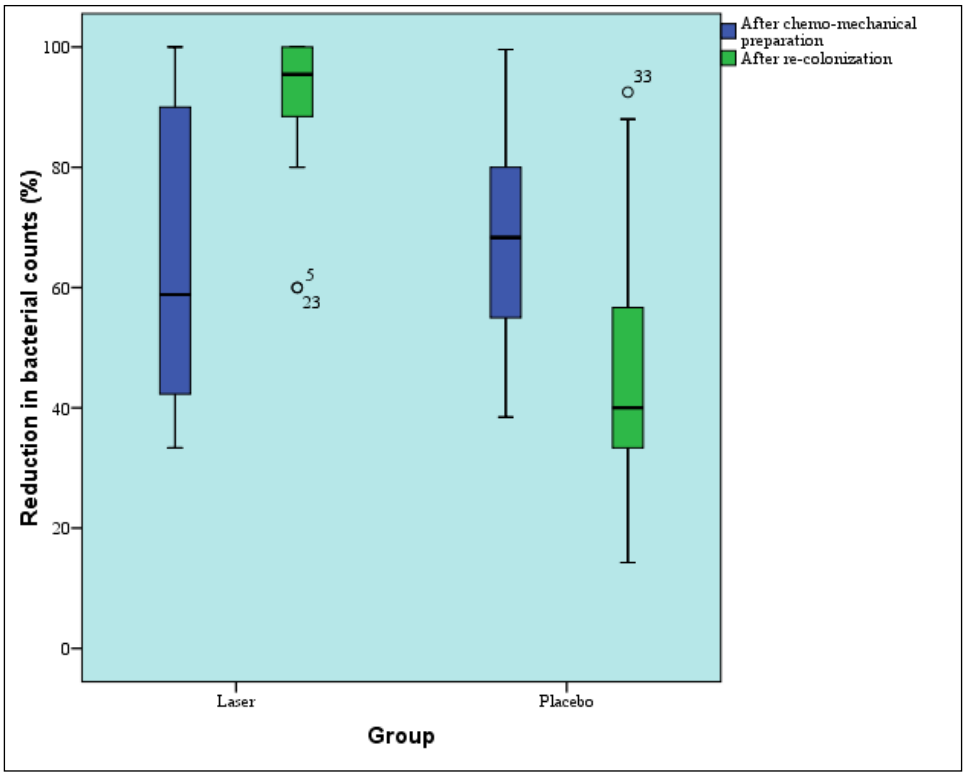

Fig. (7): Box plot representing median and range values for percentage reduction in E.faecalis counts in both groups (Circles represent outliers).