

An *In Vitro* Study Comparing the Antimicrobial Efficacy of 0.2% Chitosan, 3% Sodium Hypochlorite, 2% Chlorhexidine against *Enterococcus faecalis*, Alone and in Conjunction with Diode Laser

Pallavi Goel¹, Virat Galhotra², Sameer Makkar³, Jatinder Mohan⁴, Neetu Bala⁵, Tamanpreet Kaur⁶

ABSTRACT

The aim and objective of this study was to compare the antimicrobial efficacy of 0.2% chitosan, 3% sodium hypochlorite, 2% chlorhexidine against *Enterococcus faecalis*, alone and in conjunction with diode laser.

Materials and methods: The root canals of 72 extracted intact human single-rooted teeth with single canals were prepared, and *E. faecalis* was incubated in the root canals for 7 days. The teeth were then randomly divided into the following four experimental groups: group I: Saline, group II: 0.2% Chitosan, group III: 3% Sodium hypochlorite, and group IV: 2% Chlorhexidine. These groups were further subdivided into three groups: (1) 10 mL irrigant only, (2) 10 mL irrigant, dried and irradiation with diode laser, (3) Diode laser was used for activation of irrigant solution. Samples were obtained from subgroups in each group and checked for turbidity. The effect of each irrigant was evaluated by counting the number of colony-forming units observed on inoculation with samples taken from the irrigated canal on bile esculin azide agar. The data thus obtained was recorded and put to statistical analysis.

Results: Significant reductions were noted in *E. faecalis* colony counts in all groups ($p < 0.05$). The greatest reduction in colony count (0%) was noted in group IV followed by group II. Also, samples disinfected with diode laser after root canal irrigation showed less number of colony-forming units per mL as compared to the samples irrigated with root canal solutions alone or diode laser alone.

Conclusion: Chitosan has the capability for use as an accessory for disinfection of the root canal system. The application of an 810-nm diode laser by itself did not have the adequate antimicrobial activity to be used as an adjunct in root canal therapy. Irradiation with diode laser ought to be used in conjunction with the irrigant to gain maximum antibacterial effect against *E. faecalis*.

Keywords: Antimicrobial efficacy, Chitosan, Chlorhexidine, Diode laser, Sodium hypochlorite.

International Journal of Clinical Pediatric Dentistry (2022): 10.5005/jp-journals-10005-2351

INTRODUCTION

A comprehensive approach to debridement and disinfection of the pulpal space is deemed essential for long-term success of endodontic treatment.¹ It is still possible for residual pulpal tissue, bacteria, and dentin debris to persist in the irregularities of the root canal after scrupulous mechanical preparation. Therefore, irrigation is considered a vital component of biomechanical preparation.²

Sodium hypochlorite solution has the potential to dissolve tissue, restrain bacterial growth, and there is no clinical toxicity, when appropriately utilized.³ CHX molecule has a positive charge and consists of a hydrophobic as well as lipophilic component. Its effectiveness is based on the interaction of the positive charge of the molecule on the phosphate groups on microbial cell walls which are negatively charged, thereby altering the osmotic balance of the cells.⁴ It is less caustic, has substantivity, has broad-spectrum antibacterial effects, and is recommended in retreatment cases.⁵

These root canal irrigating solutions fulfill a few ideal prerequisites as they have a broad antimicrobial spectrum, can dissolve necrotic pulp tissue, inactivate endotoxins, and either forestall the development of the smear layer or dissolve it once it has formed. However, there are some drawbacks associated with these irrigating solutions.⁵

The structural properties of dentin can be altered by chelating agents which results in an impaired mechanical integrity and an increased likelihood of bacterial attachment to the collagen.

^{1,2}Department of Dentistry, All India Institute of Medical Sciences, Raipur, Chhattisgarh, India

^{3,5,6}Department of Conservative Dentistry and Endodontics, Swami Devi Dyal Hospital and Dental College, Barwala, Haryana, India

⁴Department of Dentistry, University of Colorado School of Dental Medicine, Aurora, Colorado, USA

Corresponding Author: Pallavi Goel, Department of Dentistry, All India Institute of Medical Sciences, Raipur, Chhattisgarh, India, Phone: +91 8360895507, e-mail: drpallavigoyal22@gmail.com

How to cite this article: Goel P, Galhotra V, Makkar S, et al. An *In-vitro* Study Comparing the Antimicrobial Efficacy of 0.2% Chitosan, 3% Sodium Hypochlorite, 2% Chlorhexidine against *Enterococcus faecalis*, Alone and in Conjunction with Diode Laser. *Int J Clin Pediatr Dent* 2022;15(1):109–114.

Source of support: Nil

Conflict of interest: None

Utilization of high concentration of NaOCl solution for extended time duration results in ultrastructural damage into the dentin. Furthermore, complete debridement of the root canals and eradication of the biofilm bacteria is not possible with NaOCl solution.

Therefore, to overcome the perceived limitations of these irrigating solutions research continues to find better irrigants for the endodontic procedure which are capable of cleaning dentinal

tubules effectively by removing the smear layer along with the debris and necrotic tissue of the canal.

Chitosan is usually attained by alkaline deacetylation from chitin, which is a predominant component of crustacean exoskeletons. It is a non-toxic cationic biopolymer that has been proposed to induce the remineralization of the exposed and demineralized dentin structure *via* covalent immobilization of chitosan on dentinal collagen. It could be attributed to its functional phosphate groups which might hold together securely with calcium ions; thus, forming a conducive surface for crystal nucleation, resulting in the formation of a calcium phosphate layer.⁶ Chitosan, given its antimicrobial properties, biocompatibility and lack of toxicity has gotten a lot of heed in dental research.⁷

Completed canal sterilization is possible with laser light as it can reach up to >1000 µm into the dentin. In recent years, lasers application in endodontics has been investigated demonstrating their efficacy in root canal shaping and sterilization, removal of debris, and sealing of dentinal tubules in the root canal wall.⁸ Although lasers have been effective in their action against microbial pathogens, some controversy persists concerning the effects of different types of lasers on bacteria within the root canal. Lasers have an edge over other methods of disinfection for endodontic treatment as it reduces bacterial load, removes smear layer besides diminished apical leakage.⁹ Laser irradiation acts primarily by photothermal action.¹⁰

In untreated canals, *Enterococcus faecalis* comprises a small fraction of the flora but is a persistent organism that can give rise to peri radicular lesions.¹¹ *E. faecalis* can withstand harsh environments such as very high pH and salinity. *E. faecalis* is invulnerable to dehydration, action of bile salts, detergents, heavy metals, ethanol, azide. In persistent endodontic infections prevalence of *E. faecalis* is 24–77% which is considerably more than in primary endodontic infection (40%). It certainly can bind to dentin and invade dental tubules.¹²

To date, none of the available irrigants have the ideal requisites for attaining successful treatment outcome. Studies have tried to evaluate different permutations and combinations of irrigating solutions to achieve better results but so far there are limited studies on concomitant use of chitosan and diode laser. Thus, this study intends to evaluate the antimicrobial efficacy of 0.2% chitosan, 3% sodium hypochlorite, 2% chlorhexidine against *E. faecalis*, alone and in combination with diode laser.

MATERIALS AND METHODS

This study was performed on 72 single-canal freshly extracted human teeth. Radiographs from both the views, mesiodistal and labiolingual of each tooth confirmed that the tooth had a single canal. Working length was measured from the coronal reference point to 1 mm short of the apical foramen. Root canals were prepared till #30 file using K3 rotary files. While using rotary files, canals were rinsed with distilled water. The teeth were then transferred into microtubes and autoclaved twice at 121°C for 20 minutes. The sterility of the sample was verified by immersing each sample in a test tube containing 5 mL of freshly prepared sterile brain heart infusion broth (BHI) incubated at 37°C for 48 hours. If no turbidity occurred, the samples were considered as sterile but if it became turbid, the sample was sterilized again.

Bacterial Inoculation of Root Canals

E. faecalis (MTCC 2729 equivalent to ATCC10100) in the freeze-dried form was first inoculated in tryptone soy broth. For confirmation, bile esculin azide agar was used to inoculate the culture at 37°C for 24 hours. *E. faecalis* showed Black color colonies on bile esculin azide agar. Each sample was immersed into a test tube containing 5 mL of sterile brain heart infusion broth. These test tubes containing the samples were contaminated. *E. faecalis* colonies were picked up with an inoculating loop from plates of bile esculin azide agar, dissolved in test tubes containing the samples and incubation was done at 37°C for minimum seven days (Figs 1 and 2).

Checking for Turbidity

The infected samples had been out from the turbid brain heart infusion broth and rinsed with freshly prepared distilled water. The external surface of the sample was wiped with gauze dipped in alcohol. The samples had been then dipped into fresh sterile BHI broth and incubated at 37°C. The appearance of turbidity indicated that the samples were infected with *E. faecalis*. In no turbidity appeared, they were reinfected, and the complete procedure was repeated again until the samples gave positive results (Figs 3 and 4).

Experimental Procedures

The samples ($n=72$) thus prepared were allocated to four equal groups as follows:



Fig. 1: *Enterococcus faecalis* colonies on bile esculin azide agar



Fig. 2: Sterile tooth dipped in brain heart infusion broth

Group I a: The prepared infected canal was irrigated passively with 2 mL of saline with the help of a side vented needle inserted until slightly short of middle third of the canal. The solution was left in the canal for 2 minutes and this procedure was repeated for four more times, the total treatment time was 10 minutes, and the solution was 10 mL. Then the sample was dabbed in dry sterile gauze, and the canals were dried with the sterile paper points which were then immersed in sterile brain heart infusion broth and incubation was done for 72 hours at 37°C. Group I b: Canal rinsed with 10 mL saline and then dried using paper points. A diode laser with a wavelength of 810 nm was used to irradiate the dried root canals with a 200 µm endodontic tip. The fiber tip was inserted into the canal 1 mm short of the apex for 5 seconds and withdrawn coronally with helical movement at 1 or 2 mm/ seconds and this procedure was done for 4 more times, following which sample was taken from dried canals using wet paper points which were then immersed in sterile brain heart infusion broth and incubated for 72 hours at 37°C. Group I c A diode laser with a wavelength of 810 nm was used to irradiate the canals using 200 µm endodontic tip. 2 mL of Saline was applied and activated for 5 seconds using an endodontic fiber tip placed into the canal 1 mm short of the apex and withdrawn coronally with helical movement at 1 or 2 mm/seconds. This procedure was done four

more times to a total of 10 mL solution. Then the sample was dabbed in dry sterile gauze, and the canals were dried with the sterile paper points which were then immersed in sterile brain heart infusion broth and incubated for 72 hours at 37°C. Similarly, procedure was done in group II, III, IV using 0.2% chitosan, 3% sodium hypochlorite and 2% chlorhexidine, respectively (Figs 5 to 7).

Checking for Disinfection of Samples

Sample paper points were placed in a test tube containing BHI broth, incubated at 37°C for 48 hours and turbidity was checked by comparing with a test tube containing the uninfected samples dipped in BHI broth.

Antimicrobial Assessment After Irrigation (Quantitative Analysis of *Enterococcus faecalis*)

All the samples whether it showed turbidity or not were serially diluted five times to obtain the final suspension. 1 mL of suspension from the last dilution was inoculated on bile esculin azide agar with a micropipette and incubated at 37°C for 24 hours. The plates were then observed for growth or no growth. Colony-forming units were obtained from the plates in which growth was observed by dilution plate method.

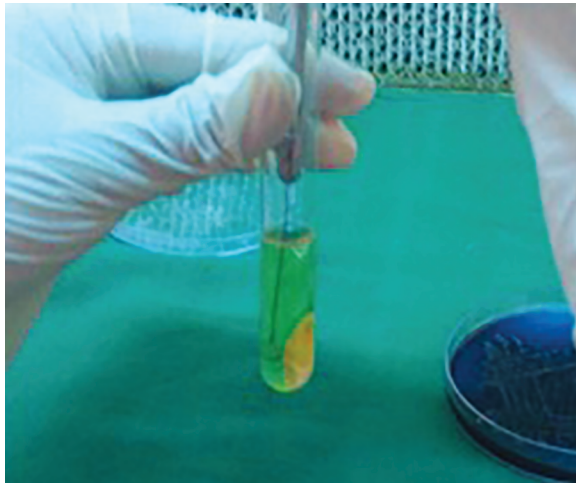


Fig. 3: Contamination of brain heart infusion broth with *Enterococcus faecalis*



Fig. 5: Turbidity compared with a test tube containing 5 mL brain heart infusion broth



Fig. 4: Presence of turbidity indicated contamination with *Enterococcus faecalis*



Fig. 6: Diode laser was used to irradiate the dried canal

Statistical Analysis

The effectiveness of each irrigant was assessed by counting the number of colony-forming units observed when the sample from the canal was inoculated on bile esculin azide agar. The data thus obtained was recorded and put to statistical analysis using non-parametric Kruskal–Wallis test followed by Mann–Whitney test for comparison of subgroups a, b, c within each group.

RESULTS

The results of the present study showed that number of bacterial colonies varied from irrigant to irrigant and no growth was observed in samples irrigated with 2% chlorhexidine. Growth was observed in 40 samples and there was absence of growth in 32 samples. In case of saline CFU/mL varied between 1 and 125, with sodium hypochlorite CFU/mL 1–70, 0.2% chitosan between 1 and 2 and for chlorhexidine 0. When the results were put to statistical analysis, it was found out that there was a significant difference when group I (saline) was compared to group II (0.2% chitosan), group III (3% sodium hypochlorite), and group IV (2% chlorhexidine). It was observed that the maximum number of colony-forming units were observed in the group I (106.83 CFU/mL), followed by group III (54.33), group II (0.50 CFU/mL), and group IV (0 CFU/mL). As our data



Fig. 7: Turbidity in the test tubes containing sample paper points after incubation for 48 hours

was skewed, on applying nonparametric Kruskal–Wallis test followed by Mann–Whitney test it was found that there was a significant difference among the groups tested. The mean colony-forming units of all the groups in descending order is group Ia-106.83 > group Ic-56.00 > group IIIa-54.33 > group Ib- 6.83 > group IIIb-2.33 = group IIIc-2.33 > group IIa-0.50 > group IIb-0.17 > group IIc-0.17 > group IV a-0.000 = group IV b-0.000 = group IVc-0.000 (Table 1).

DISCUSSION

Unequivocal elimination of microbes from the root-canal system and forestalling reinfection leads to the perpetual success of endodontic treatment. Even after thorough biomechanical preparation irregularities of the root canal system can still harbor residual pulpal tissue, microorganisms, and dentin debris. Therefore, irrigation is considered an integral part of biomechanical preparation.²

Given the importance of efficient cleaning and elimination of microorganisms from the root canal system, the purpose of this study was to evaluate the antimicrobial efficacy of three different irrigating solutions alone and in concert with diode laser, which has the potential to be used as a supplement to mechanical debridement.

Since *E. faecalis* can resist endodontic treatment procedure, has ability to successfully colonize the root canal in a biofilm-like style and invade dentinal tubules. Hence it was selected for the purpose of this study. This bacterium is one of the intracanal bacteria which are most resistant to the actions of irrigating solutions.¹³

As per a study conducted by Barber et al., 5.25% concentration of NaOCl is the most effective among three different concentrations of 0.5%, 2.5%, and 5.25%.¹⁴ But higher concentration may cause ultrastructural damage into the dentin¹⁵ and could harm apical and periapical tissues.⁴ Therefore, we had chosen 3% concentration for our study as it is less toxic and commercially available.

Chlorhexidine has been used as an efficient substitute for NaOCl.¹³ It has a broad-spectrum antimicrobial activity against both gram-positive and gram-negative microbes. Regardless of its usefulness as a final rinse in root canals, chlorhexidine cannot be advocated as the primary irrigant in standard endodontic cases as it cannot dissolve necrotic tissue remnants and can cause discoloration of the teeth. Furthermore, side effects include loss of taste, irritation of the oral mucosa, dryness of the oral cavity, and staining of the tongue.¹⁶

Due to perceived limitations of these irrigating solutions, the development of new and better irrigating solutions for endodontic

Table 1: Comparison of mean of all the groups

Group	Subgroup	n	Mean	Std. deviation
Saline	a	6	106.83	16.750
	b	6	6.83	5.845
	c	6	56.00	10.139
2% Chitosan	a	6	0.50	0.837
	b	6	0.17	0.408
	c	6	0.17	0.408
3% Sodium hypochlorite	a	6	54.33	16.955
	b	6	2.33	1.751
	c	6	2.33	1.506
2% Chlorhexidine	a	6	0.00	0.000
	b	6	0.00	0.000
	c	6	0.00	0.000

procedures which are capable of cleaning dentinal tubules effectively by removing the smear layer along with the debris and necrotic tissue of the canal is an important concern.

Chitosan is a natural polysaccharide derived from the shells of prawns and crabs by acetylation of chitin. It is biocompatible, biodegradable, has bioadhesive properties, and is nontoxic to the human body.¹⁷

Whether the antimicrobial effect of various irrigants is because of their chemical nature or just the pure physical flushing action, saline was used as the fourth irrigant which is chemically inert and is devoid of antibacterial action.

The most of present irrigants and intracanal medicaments have a limited antibacterial spectrum and a constrained capacity to diffuse into the dentinal tubules (100 µm). Consequently, newer therapeutic strategies that penetrate up to 1110 µm should be considered to eliminate microorganisms from the root canal system. Laser light penetrates up to >1000 µm into the dentin; therefore, has a scope for complete canal sterilization.¹⁸

Limitations in laser applications can be the increase of temperature and the fact that it is not possible for laser to reach some surfaces.¹⁹ The reason for using the laser in the wet canal was to warm the irrigating solution to enhance its disinfecting effect, further laser induces cavitation, which enhance the elimination of the smear layer.²⁰

To date, none of the available irrigants have the ideal requisites for attaining successful treatment outcome. Studies have tried to evaluate different permutations and combinations of irrigating solutions to achieve better results but so far there are limited studies on concomitant use of chitosan and diode laser. The current study focused on this issue.

After 2% chlorhexidine, samples irrigated with 0.2% chitosan (group II) showed statistically least number of CFU/mL as compared to 3% sodium hypochlorite and saline. The superior performance of 0.2% chitosan in the present study may be a result of its ability to destroy bacterial adhesions thereby preventing formation of biofilms. In addition, its polycationic nature interacts with the negatively charged surface of bacteria, alters permeability of cell, and causes leakage of intracellular components. Moreover, chitosan suppresses the enzymatic degradation of bacteria thereby reducing the possibility of bacterial invasion and microfractures into dentin.

The mean colony-forming units per mL were less in subgroup b as compared to subgroup a. This may be attributed to the fact that action of root canal irrigants is confined to the outer layer of the root dentin and depth of penetration is insufficient whereas laser light penetrates the dentin up to 1000 µm or more, so it has the capacity to completely sterilize the root canal.

The results of this study show that the samples disinfected with a diode laser after root canal irrigation with chemical solutions showed a smaller number of CFU/mL as compared to the samples irrigated with root canal solutions alone or diode laser alone.

Although group I (saline) subgroup c had fewer CFU/mL than group I subgroup a, but had more colony-forming units than subgroup b. This may be attributed to the high temperature generated in dried canals which killed the bacteria and charring effect which sealed the dentinal tubules but this was not possible when diode laser was used in wet canals. Also, because warming the solution did not help as saline itself has no antimicrobial properties. But use of diode laser in wet canals in group II (0.2% chitosan) and group III (sodium hypochlorite) potentiated the effect of irrigating solutions, thereby resulting in lesser number of colonies forming units as compared to subgroup a and b.

From the results of this study, we can infer the following among the irrigants tested: 2% chlorhexidine and 0.2% chitosan have best results with least number of bacterial colonies of *E. faecalis* and should be the preferred irrigants. Diode laser increases the effectiveness of these irrigants against *E. faecalis*. It should be used in conjunction with the irrigant so as to obtain maximum antibacterial effect against *E. faecalis*. However, this is an *in vitro* study, further *in vivo* studies are needed before extrapolating these test results to the clinical scenario.

CONCLUSION

Chitosan has the capability for use as an accessory for disinfection of the root canal system. Application of an 810-nm diode laser by itself did not have adequate antimicrobial activity to be used as an adjunct in endodontic treatments. Irradiation with diode laser ought to be used in conjunction with the irrigant to gain maximum antibacterial effect against *E. faecalis*.

REFERENCES

- Gomes BP, Ferraz CC, Viana ME, et al. In vitro antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of *Enterococcus faecalis*. *Int Endod J* 2001;34(6):424–428. DOI: 10.1046/j.1365-2591.2001.00410.x
- Fidalgo TK, Barcelos R, Portela MB, et al. Inhibitory activity of root canal irrigants against *Candida albicans*, *Enterococcus faecalis* and *Staphylococcus aureus*. *Braz Oral Res* 2010;24(4):406–412. DOI: 10.1590/s1806-83242010000400006
- Macedo RG, Wesselink PR, Zaccaro F, et al. Reaction rate of NaOCl in contact with bovine dentine: effect of activation, exposure time, concentration and pH. *Int Endod J* 2010;43(12):1108–1115. DOI: 10.1111/j.1365-2591.2010.01785.x
- Mohammadi Z. Chlorhexidine gluconate, its properties and applications in endodontics. *Iran Endod J* 2008;2(4):113–125.
- Suzuki S, Masuda Y, Morisaki H, et al. The study of chitosan-citrate solution as a root canal irrigant: a preliminary report. *J Oral Hyg Health* 2014;2:142. DOI: 10.4172/2332-0702.1000142
- Perochena DC, Bramante CM, Duarte MAH, et al. Chelating and antibacterial properties of chitosan nanoparticles on dentin. *Restor Dent Endod* 2015;40(3):195–201. DOI: 10.5395/rde.2015.40.3.195
- Silva PV, Guedes DFC, Nakadi FV, et al. Chitosan: a new solution for removal of smear layer after root canal instrumentation. *Int Endod J* 2013;46(4):332–338. DOI: 10.1111/j.1365-2591.2012.02119.x
- Gunwal M, Shenoi P. Evaluation of the efficacy of 5.25% of sodium hypochlorite, 2% of chlorhexidine, MTAD and 810 diode laser in reduction of microbial count in root canal - an *in vivo* study. *J Endodontol* 2013;25:56–62.
- Kreisler M, Kohnen W, Beck M, et al. Efficacy of NaOCl/H₂O₂ irrigation and GaAlAs laser in decontamination of root canals *in vitro*. *Lasers Surg Med* 2003;32(3):189–196. DOI: 10.1002/lsm.10148
- Camargo SCC. The antibacterial effects of lasers in endodontics. *Roots* 2012;1:6–21.
- Elakanti S, Cherukuri G, Rao VG, et al. Comparative evaluation of antimicrobial efficacy of QMix™ 2 in 1, sodium hypochlorite and chlorhexidine against *Enterococcus faecalis* and *Candida albicans*. *J Conserv Dent* 2015;18(2):128–131. DOI: 10.4103/0972-0707.153067
- Hedge V. *Enterococcus faecalis*: clinical significance and treatment considerations. *Endodontology* 2009;21:48–52.
- Jeanson MJ, White RR. A comparison of 2.0% chlorhexidine gluconate and 5.25% sodium hypochlorite as antimicrobial endodontic irrigants. *J Endod* 1994;20(6):276–278. DOI: 10.1016/s0099-2399(06)80815-0
- Berber VB, Gomes BP, Sena NT, et al. Efficacy of various concentrations of NaOCl and instrumentation techniques in reducing *Enterococcus faecalis* within root canals and dentinal tubules. *Int Endod J* 2006;39(1):10–17. DOI: 10.1111/j.1365-2591.2005.01038.x

15. Zhang K, Kim YK, Cadenaro M, et al. Effects of different exposure times and concentrations of sodium hypochlorite/ethylenediaminetetraacetic acid on the structural integrity of mineralized dentin. *Endodontology* 2010;36(1):105–109. DOI: 10.1016/j.jjoen.2009.10.020
16. Gomes PFA, Vianna M, Zaia AA, et al. Chlorhexidine in endodontics. *Braz Dent J* 2013;24(2):89–102. DOI: 10.1590/0103-6440201302188
17. Berástegui E, Molinos E, Ortega J. To comparison of standard and new chelating solutions in endodontics. *J Dent Sci* 2017;2(2):1–8. DOI: 10.23880/oajds-16000131
18. Stevanovic M, Petrovska M, Stevanovic M, et al. Bactericidal effects of Er:YAG laser irradiation in root canals. *J Oral Laser Appl* 2004;4: 43–46.
19. Asnaashari M, Godiny M, Azari-Marhabi S, et al. Comparison of the antibacterial effect of 810 nm diode laser and photodynamic therapy in reducing the microbial flora of root canal in endodontic retreatment in patients with periradicular lesions. *J Lasers Med Sci* 2016;7(2):99–104. DOI: 10.15171/jlms.2016.17
20. Zohair AE, Fouad R. Synopsis is using dental diode laser in endodontic. *EC Dent Sci* 2016;5:1139–1144.