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Scanning electron microscopic evaluation of the dentinal tubule occlusion using diode laser 976 nm and 0.5% sodium flouride gel: An *In vitro* study

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Abstract
Background: Dentin hypersensitivity (DH) is a painful condition associated with challenging treatment options.
Aims: This study aimed to evaluate the effectiveness of a diode laser (976 nm) for occluding dentinal tubules *in vitro* and to compare the effectiveness of this laser with that of the conventional chemical agent sodium fluoride gel in occluding the Dentinal Tubules.
Materials and Methods: Ten extracted human premolars were utilized in the current *in vitro* experimental scanning electron microscopic (SEM) study. Using a diamond saw with coronal dentin from each tooth specimen, dentin disks 1.0 mm in thickness were cut perpendicularly to the long axis of each tooth. Ten dentin disks were prepared, and each specimen was divided into four equal parts, pretreated with ethylenediaminetetraacetic acid (17%), and subsequently divided into four groups: (Group 1) the control group, (Group 2) the 976 nm diode laser with a power of 1 watt, (Group 3) the sodium fluoride only group and (Group 4) the combined laser and sodium fluoridearnish group. The samples were analysed using a scanning electron microscope at different magnifications (100 µm, 50 µm, 20 µm, and 2 µm). ANOVA was used to compare the differences in dentinal tube diameter among the four groups, with the level of significance set at a P value <0.05.
Results: Group 1 (control, no treatment) had all the dentinal tubules opened. Both the laser group and the sodium fluoride group demonstrated partial occlusion and narrowing of most of the dentinal tubules, while the combined group showed total occlusion of almost all of the dentinal tubules. There was a statistically significant difference between the four groups regarding the diameter of the dentinal tubules.
Conclusions: The combination of a diode laser (976 nm), 1 W, and sodium fluoride varnish significantly affected dentinal tubule occlusion, thus preventing DH.

Keywords: Dentin hypersensitivity, diode laser, desensitizing agents, scanning electron microscope

Introduction
Dentin Hypersensitivity DH is one of the most painful dental conditions caused by extrinsic stimuli on the exposed dentin surface [1]. The incidence of DH has been reported to range from 8–35% of individuals depending on the population samples studied and it is more common in women than in men [2]. Sodium fluoride gel (NaF), which is a tubule-occluding agent, is the most commonly used agent for the treatment of DH through the precipitation of calcium fluoride in the tubules [3, 4, 5]. Covering the dentinal tubules with composite restoration, glass ionomer cement, periodontal graft, or crown placement is another effective treatment [6]. Lasers are promising alternative treatments for DH that have revolutionized the dental field [7]. The exact mechanism of action of lasers in DH is not completely understood. Several theories, such as photobiomodulation of odontoblastic cells, elimination of dentinal tubules, thermomechanical ablation of dentinal fluid, and blockade of C and Aβ nerve fibres, have been proposed [8, 9]. Using laser irradiation combined with chemical agents such as sodium fluoride and stannous fluoride can enhance treatment efficacy compared to using a laser alone [10] Inspite of many treatment modalities that are used in the treatment of DH by occluding dentinal tubules, such as lasers or conventional desensitizing agents, the combination of the laser and the desensetized agent (Sodium Flouride varnish) should be investigated more. The objective of this study was to assess and compare the Occluding effect of a 1-Watt diode laser (976 nm) and sodium fluoride varnish on dentinal tubules by scanning electron microscopy (SEM).

Materials and methods
Experimental *in vitro* SEM study (posttest design). A total of ten sound premolars extracted for orthodontic reasons were collected for this study. These premolars were collected from orthodontics clinics in Khartoum State, autoclaved at 121°C and 15 lbs psi for 20 min, and stored in deionized water. The study was conducted at the Laser Institute, Sudan University of Science and Technology (SUST). The sample size was determined according to the study's power of 86% (0.86) [11]. Coronal dentin disks 1.0 mm in thickness were cut perpendicular to the long axis of each Specimen at a level 1.0 mm above the cementoenamel junction with a diamond saw using a low motor headpiece (Southeast Dental Supply, Japan). To determine the surface morphological changes in the dentin samples, a central area 5×5 mm in diameter on the dentin disks was the experimental area. Dentin specimens were selected; each

specimen was divided into four equal parts for control and testing. All four parts were immersed with ethylenediaminetetraacetic acid (EDTA) (Enpunuo EDTA Root Canal Lubricant, China) for 1 min and then cleaned with distilled water for 30 seconds [12] The study specimens were devised into four groups as follows: Group 1, control no treatment; Group 2, treated with a 976 nm diode laser(SOLASE dental diode laser, LAZON, China); Group 3, Specimens were polished with sodium fluoride(Enamelast fluoride varnish, SX-ultradent, China); and Group 4, treated with a combination of laser and sodium fluoride varnish. Notably, Group 3 and Group 4 were irradiated three times—during the second and third sessions (24 and 48 hours, respectively) [13]. After the initial session—for a duration of 20 seconds with a 976-nm diode laser and a power of 1 Watts [13]. The control (samples were used without any further treatment), the second group was treated with NaF only, and the third group was irradiated with a diode laser device [wavelength: 976 nm; power: 1 W; irradiation time: 20 s; irradiation mode: continuous pulse mode; distance: 1 cm; fibre delivery system: 400 µm; noncontact mode] [13]. While the last group was first exposed to laser radiation for 20 and, Naf was subsequently applied for 4 minutes according to the manufacturer's directions. To detect the effects of the diode laser and sodium fluoride varnish on the dentinal tubules, all the samples were analysed at room temperature using a scanning electron microscope (SEM) (Jeol JSM 840-A, Japan). The specimens were primarily dehydrated in blue silicon and attached to aluminum stubs. Different magnifications (100 µm, 50 µm, 20 µm, and 2 µm) were observed from the center of each sample in each of the four groups [14].

The study was approved by the Ethical Committee of the Sudan University of Sciences and Technology (SUST), Institute of Laser. Patients were asked to leave their teeth voluntarily for the purpose of the study, and those who agreed signed an informed written consent.

The data were analysed using SPSS, version 21 (Chicago, Illinois, USA). Based on the studied groups, descriptive statistics (mean and standard deviation) of the diameter of the tubules and the area were computed (Figure 1). T tests and ANOVAs were used to compare different parametric data. A P value <0.05 indicated a statistically significant difference.

Results

Scaning electron microscopic pitures showed was a change in the surface morphology of the dentinal tubules among the four groups, with Group 1 (control- no treatment) demonstrating the opening of all the dentinal tubules (Group 1 in Figure 2). In contrast, both Group 2 (diode laser alone) and Group 3 (sodium fluoride-alone) exhibited partial occlusion and narrowing of most of the dentinal tubules. Compared with Group 2, Group 3 exhibited greater occlusion, (Fig. 2). All the dentinal tubules in Group 4 were nearly occluded.

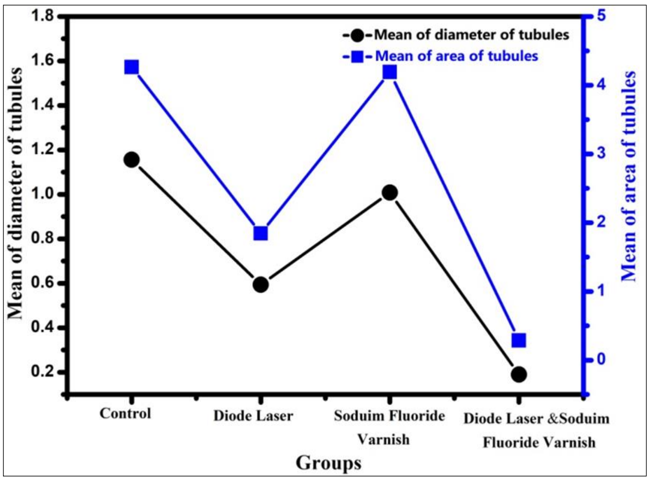
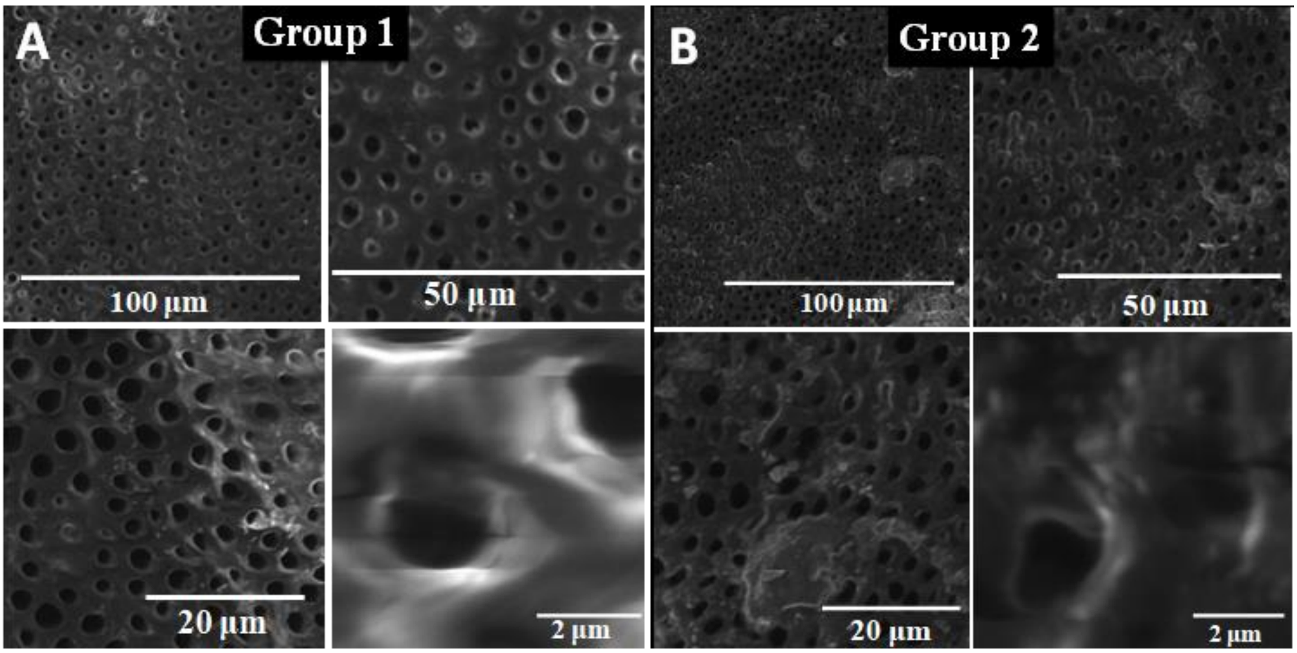


Fig 1: The mean diameter of tubules and mean area of tubules for the studied groups



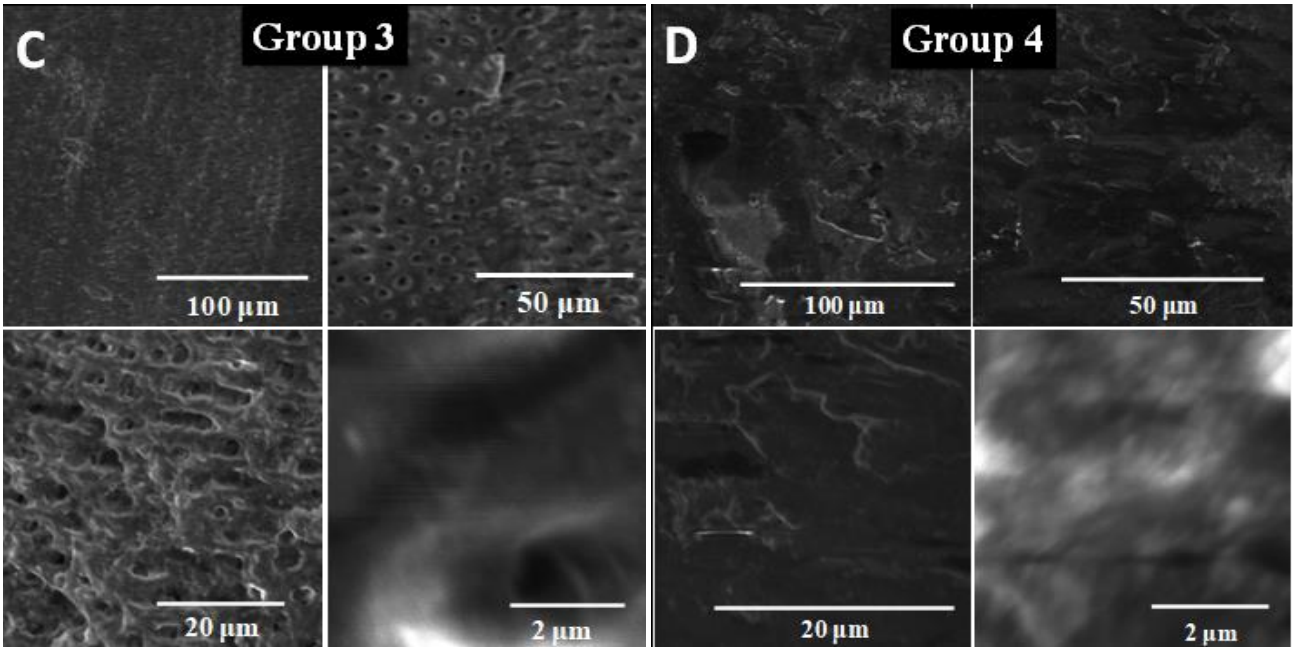


Fig 2: SEM images of each sample in the studied groups at different magnifications

A comparison of the four groups in terms of the diameter and width of the dentinal tubules revealed a statistically significant difference between Group 1 and Group 4, with a p value of 0.016. There was a statistically significant difference between Group 3 and Group 4 (p=0.044). There were statistically significant differences in the diameter of the 5×5 mm area on the dentin disks between Group 1 and Group 4 (p=0.024). In contrast, statistically significant differences between Group 3 and Group 4 were shown, with a p value of 0.027. Analysis of variance (ANOVA) was performed for the variables according to the studied groups (TEST) to compare the diameter of the tubules and area among the four groups. The significance values were P=0.014 for the diameter of the tubules and P=0.013 for the area; both of these values are <0.05, indicating that there are statistically significant differences between the groups. Therefore, the null hypothesis was rejected, and the alternative hypothesis was accepted.

Discussion

Treatment of DH is challenging, and occlusion of the open tubules should be the focus, reducing the flow of fluid within the dentinal tubules and subsequently reducing pain transmission [15]. Treatment options for DH include the use of a specific toothpaste, therapy with laser therapy, fluoride therapy, desensitizers, gingival surgery, or restorations [16]. Several previous studies have used higher energy (1 W) densities to provoke dentinal melting, leading to occlusion of the dentinal tubules; if the laser parameters are well adapted, this could reduce the thermal effects that can be produced [17, 18]. Changes in the dentinal tubules at the microscopic level [14] were detected by scanning electron microscopy (SEM) analysis of the samples [14].

The difference between the results for Group 2 (diode laser-alone) and Group 1(control- no treatment) is consistent with the study conducted by Garcia *et al.* [19]. In addition, Ying *et al.* reported the sealing of exposed dentinal tubules with melted and recrystallized dentine due to the thermal and antagonistic effects of the laser [20]. This may be due to the absorption of energy from the 976-nm diode laser by the minerals present in the dentine, which results in the melting and formation of crystallized dentine.

The dentinal tubule openings became narrower due to calcium fluoride crystal deposition, which agrees with the findings reported by Absidais *et al*. [21]. The sodium fluoride group exhibited partial occlusion and narrowing of most of the dentinal tubules which is similar to the findings of a study by Kunam *et al*. who reported that sodium fluride caused narrowing of the dentinal tubular lumen but failed to produce complete tubular occlusion. This finding can be explained by the formation of calcium fluoride crystals due to the chemical reaction between fluoride in the sodium fluoride varnish and the calcium ions in the dentinal fluidMoreover, the size of the formed crystals was very small (0.05 µm) and insufficient to cause total occlusion of the dentinal tubules, which is why only partial occlusion of the tubules was observed [22]. In another study, IshaSuri reported that the number and width of DTs in hypersensitive-exposed areas are greater than those in normal dentine areas, necessitating a more prolonged treatment to ensure total occlusion of the tubules. [23].

The result of combination of a diode laser and sodium fluoride varnish aligns with the findings of several studies that demonstrated the synergistic effect of lasers in conjunction with chemical agents [24, 25, 26, 27]. The total occlusion of almost all the dentinal tubules, with the formation of a homogeneous layer covering the surface of the dentinal tubules; may be due to the increased adhesion of sodium fluoride varnish to tubules when sodium fluoride is combined with a diode laser, According to the discussion above, it can be concluded that the combination of laser treatment and sodium fluoride varnish yielded superior results in comparison with those of Group 2 (diode laser-alone) and Group 3 (sodium fluoride-alone).

Limitations

This study has several potential limitations. First, the use of a larger sample size may yield a more accurate findings.

Second, the teeth used in the study were extracted from different patients, which may act as a confounding factor in relation to the minerals, context, and physiological occlusion of the dentinal tubules by the formation of sclerotic dentin. The last point one technique was used to demonstrate that the application of a laser is effective at occluding Dentinal tubules, further studies for comparison of other materials also different power of lasers should be conducted.

Conclusion

In this study, the best effect in occluding the dentinal tubules were achieved by diode laser and sodium fluoride varnish treatment (Group 4). The high adhesion of sodium fluoride varnish to the dentinal tubules when combined with laser energy likely contributed to the superior efficacy of the combined treatment.

Declarations:

Ethics approval and consent to participate: An ethical approval certificate was obtained from the Ethical Committee of the Sudan University of Sciences and Technology (SUST). Patients were asked to give up their teeth voluntarily, and those who agreed signed an informed written consent.

Experimental method statement: All the experiments and methods were performed in accordance with the relevant guidelines and regulations. All the experimental protocols were approved by the Ethics Committee of the Sudan University of Sciences and Technology (SUST).

Consent for publication: Not applicable.

Availability of data and materials: The data that support the findings of this study are available from the corresponding author [Reem Esam Siddig] upon reasonable request.

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Authors' contributions

RES: Literature search, Experimental studies, Data acquisition, Manuscript preparation.

AEA: Concept, Manuscript editing, Manuscript review.

EMA: Design, Manuscript editing, Manuscript review.

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References

1. Mason S, Burnett GR, Patel N, Patil A, Maclure R. Impact of toothpaste on oral health-related quality of life in people with dentine hypersensitivity. BMC Oral Health,2019:19:226.
2. Guerra F, Corridore D, Cocco F, Arrica M, Rinaldo F, Mazur M, *et al*. Oral health sentinel-based surveillance: A pilot study on dentinal hypersensitivity pain. Clin Ter,2017:168–e337.
3. Vatturu S, Ganugapanta VR, Teja NR, Singaraju GS, Mandava P, Priyanka JY. Comparative evaluation of the efficacy of the desensitizing and remineralizing agent in the reduction of dentin hypersensitivity after orthodontic debonding—a randomized clinical trial. Med Pharm Rep,2021:94:229–238.
4. Kiesow A, Menzel M, Lippert F, Tanzer JM, Milgrom P. Dentin tubule occlusion by a 38% silver diamine fluoride gel: An *in vitro* investigation. BDJ Open,2022:8:1–5.
5. Viswanath N, Inbaraj AS, Amaechi BT, Gandhi GD, Subramani RP. Influences of desensitizing agents on bond strength of etch-and-rinse and self-etch adhesive system to dentin. J Conserv Dent,2020:23:522–527.
6. Clark D, Levin L. Non-surgical management of tooth hypersensitivity. Int Dent J,2016:66(5):249-56. doi: 10,1111/idj,12247. Epub 2016 Jun 15. PMID: 27301300: PMCID: PMC9376650.
7. Thermo Fisher Scientific Inc. Electron Microscopy Solutions. Available from: https://www.fei.com/products/sem/quanta-sem/. Accessed April 28, 2018.
8. Namour A, Nammour S, Peremans A, *et al*. Treatment of dentinal hypersensitivity by means of Nd: YAP Laser: a preliminary *in vitro* study. Sci World J,2014:2014:323604.
9. Sgolastra F, Petrucci A, Gatto R, *et al*. Effectiveness of laser in dentinal hypersensitivity treatment: a systematic review. J Endod,2011:37:297–303.
10. Raut CP, Sethi KS, Kohale B, Mamajiwala A, Warang A. Evaluation of diode laser and stannous fluoride in the treatment of root sensitivity after access flap surgery: Randomized controlled clinical trial. J Indian Soc Periodontol,2018:22(2):158-163. doi: 10.4103/jisp.jisp_2_18. PMID: 29769771: PMCID: PMC5939024.
11. Available from: https://onlinestatbook.com/2/calculators/power_calc.html. Accessed June 3, 2022.
12. Tian L, Peng C, Shi Y, Guo X, Zhong B, Qi J, *et al*. Effect of mesoporous silica nanoparticles on dentinal tubule occlusion. An *in vitro* study using SEM and image analysis. Dent Mater J,2014:33(1):125-32.
13. Tabibzadeh Z, Fekrazad R, Esmaeelnejad A, Shadkar MM, Khalili Sadrabad Z, Ghojazadeh M. Effect of combined application of high- and low-intensity lasers on dentin hypersensitivity: A randomized clinical trial. J Dent Res Dent Clin Dent Prospects,2018:12(1):49-55.
14. Oak A. Effect of Diode Laser on Sealing Ability of Dentinal Tubules – An *In vitro* SEM Study. Natl J Integr Res Med [Internet],2018 Jul 24 [cited 2023 Dec 25]:9(3):48-51. Available from: https://nicpd.ac.in/ojs-/index.php/njirm/article/view/2353.
15. Chowdhary Z, Gupta P, Kaur J, Garg Y, Swarup NJ. Multifaceted assessment of dentine hypersensitivity, evaluation of demographic prevalence along with associated factors: A cross-sectional study. Indian Soc Periodontol,2019:23(1):64-68.

16. Marto CM, Baptista Paula A, Nunes T, Pimenta M, Abrantes AM, Pires AS, *et al*. Evaluation of the efficacy of dentin hypersensitivity treatments—A systematic review and follow-up analysis. Oral Rehabil,2019:46(10):952-990.
17. Namour A, Nammour S, Peremans A, Heysselaer D, De Moor RJ. Treatment of dentinal hypersensitivity by means of Nd: YAP Laser: a preliminary *in vitro* study. Sci World J,2014:2014:323604. doi: 10,1155/2014/323604. Epub 2014 Oct 14. PMID: 25383368: PMCID: PMC4212543.
18. Umberto R, Claudia R, Gaspare P, Gianluca T, Alessandro del V. Treatment of dentine hypersensitivity by diode laser: a clinical study. Int J Dent,2012:2012:858950. doi: 10,1155/2012/858950. Epub 2012 Jun 25. PMID: 22792109: PMCID: PMC3389731.
19. Garcia Lda F, Naves LZ, Farina AP, Walker CM, Consani S, Pires-de-Souza Fde C. The effect of a 980 nm diode laser with different parameters of irradiation on the bond strength of fiberglass posts. Gen Dent,2011:59(1):31-7:38-9, 80.
20. Liu Y, Gao J, Gao Y, Xu S, Zhan X, Wu B. *In vitro* Study of Dentin Hypersensitivity Treated by 980-nm Diode Laser. J Lasers Med Sci,2013:4(3):111–119.
21. Absi EG, Addy M, Adams D. Dentin hypersensitivity: A study of the patency of dentinal tubules in sensitive and nonsensitive cervical dentin. J Clin Periodontol. 1987:14(5):280-5.
22. Kunam D, Manimaran S, Sampath V, Sekar M. Evaluation of dentinal tubule occlusion and depth of penetration of nano-hydroxyapatite derived from chicken eggshell powder with and without addition of sodium fluoride: An *in vitro* study. J Conserv Dent,2016 May-Jun:19(3):239-44. doi: 10.4103/0972-0707,181940. PMID: 27217637: PMCID: PMC4872578.
23. Suri I, Singh P, Johar Shakir Q, Shetty A, Bapat R, Thakur R. A comparative evaluation to assess the efficacy of 5% sodium fluoride varnish and diode laser and their combined application in the treatment of dentin hypersensitivity. J Indian Soc Periodontol,2016:20(3):307–314.
24. Umberto R, Claudia R, Gaspare P, Gianluca T, Alessandro del V. Treatment of dentine hypersensitivity by diode laser: a clinical study. Int J Dent,2012:2012:858950.
25. Yilmaz HG, Kurtulmus-Yilmaz S, Cengiz E. Long-term effect of diode laser irradiation compared to sodium fluoride varnish in the treatment of dentine hypersensitivity in periodontal maintenance patients: a randomized controlled clinical study. Photomed Laser Surg,2011:29(11):721-5.
26. Dantas EM, Amorim FK, Nóbrega FJ, Dantas PM, Vasconcelos RG, Queiroz LM. Clinical efficacy of fluoride varnish and low-level laser radiation in treating dentin hypersensitivity. Braz Dent J,2016:27:79-82.
27. El Mobadder M, Namour A, Namour M, *et al*. Dentinal hypersensitivity treatment using diode laser 980 nm: *In vivo* study. Dent J (Basel),2019:7(1):5.