"COMPARATIVE ASSESSMENT OF DIODE LASER, COMMERCIALLY AVAILABLE TOOTHPASTE CONTAINING DESENSITIZING AGENT AND COMBINATION OF BOTH ON THE OCCLUSION OF DENTINAL TUBULES USING SCANNING ELECTRON MICROSCOPE – AN INVITRO STUDY"

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REG NO. IK0220005

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Professor and Head
Department of Periodontics
KLE V.K. Institute of Dental Sciences
Belagavi

Dr. VINAYAK KUMBHOJKAR *M.DS*
Professor & Head,
Department of Periodontics,
KAHER's KLE V. K. Institute of Dental Sciences, Belagavi

Date: 27/12/2022
Place: Belagavi

Dr. ALKA KALE *M.D.S, PhD.*
Principal,
KAHER's KLE V. K. Institute of Dental Sciences, Belagavi

PRINCIPAL
KLE V.K. Institute of Dental Sciences
Nehru Nagar, BELAGAVI-590010.

Date: 27/12/2022
Place: Belagavi

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LIST OF ABBREVIATIONS

SEM	Scanning Electron Microscope
DH	Dentinal Hypersensitivity
T	Toothpaste
5L	Laser
T+L	Toothpaste + Laser
CSPS	Calcium sodium phosphosilicate
DT	Dentinal tubules
GaAlAs	Gallium-Aluminum-Arsenide laser
ANOVA	Analysis of Variance
T-HSD Test	Tukey's Honest significant Differences
kx	Magnification thousand times
µm	Micrometer
mm	Millimeter

ABSTRACT

BACKGROUND: Dentinal hypersensitivity is defined as an abnormal response of the exposed vital dentine to thermal, chemical, or tactile stimuli. The treatment is usually topical application of desensitizing toothpastes, varnishes, fluoride iontophoresis, remineralizing agents, anti-inflammatory agents, agents that block the neuronal response, and low input laser irradiation. NovaMin® is a calcium sodium phosphosilicate based material designed to remineralize the enamel and give immediate and long-lasting relief from tooth sensitivity. GaAlAs Diode laser (940nm) have the ability to melt peritubular dentin, can occlude dentinal tubules partially or totally, and therefore reduce hypersensitivity symptoms. These desensitizing agents when exposed to saliva release mineral ions that become available to the natural remineralization process in the mouth.

Hence, this study was undertaken to assess and compare the effect of diode laser, commercially available toothpaste containing desensitizing agent and combination of diode laser along with commercially available toothpaste containing desensitizing agent on dentinal tubules using scanning electron microscope.

OBJECTIVE: To assess and compare the effect of diode laser (940nm), commercially available toothpaste containing desensitizing agent and combination of diode laser (940nm) along with commercially available toothpaste containing desensitizing agent on dentinal tubule occlusion using scanning electron microscope.

MATERIALS AND METHODS: 120 human dentin discs of diameter 5 mm and thickness of 2 mm were sanded with wet 600-grit carborundum paper and treated with 0.5M EDTA. Specimens were allotted to three groups of twenty each: Group 1[C]-

Control group, Group 2[T]–NovaMin® containing dentifrice application, Group 3[L] – Diode laser and Group 4[T+L]- containing combination of NovaMin® containing dentifrice and diode laser (940nm). The percentage of occluded tubules in the four groups was calculated, and analyzed and the same dentin discs were kept in citric acid for 5 mins and again the percentage occlusion of dentinal tubules was calculated.

RESULTS: Both NovaMin®, Diode laser (940nm) and combination of NovaMin® and Diode laser (940nm) demonstrated occlusion of tubules more than that of control group both before and after citric acid challenge. The percentage of occlusion was more with diode laser than NovaMin®, even after citric acid challenge. The maximum amount of tubules occlusion was seen with combination of NovaMin® toothpaste and diode laser 940nm even after citric acid challenge.

CONCLUSIONS: Although dentinal tubules occlusion was effective by both NovaMin® & Diode laser (940nm), the combination of both NovaMin® & Diode laser (940nm), produced maximum amount of occlusion of dentinal tubules and seemed to be most effective for dentinal hypersensitivity in dentin disc model.

KEYWORDS: Dentin hypersensitivity, Diode laser, Dentinal tubule occlusion, Scanning electron microscope, calcium sodium phosphosilicate, Gallium-Aluminum-Arsenide laser.

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INTRODUCTION

"Dentinal Hypersensitivity" (DH) as defined by the world workshop is distinguished by a short, sharp pain originating from exposed dentin in response to stimuli, typically thermal, evaporative, tactile, osmotic or chemical and which cannot be ascribed to any other dental defect or disease.[1]

DH can affect people of any age and gender. It has a slightly higher incidence in females than males and mostly affects the age group, ranging from 20-50 years. The incidence is substantially higher (60-90%) in patients having periodontal disease.[2,3]

Dentinal exposure occurs as a result of gingival recession caused by wasting diseases such as attrition, abrasion, abfraction and erosion, periodontal disease, excessive flossing, pocket reduction surgery, or faulty toothbrushing habits leading to removal of the smear layer, thereby opening the tubular plugs causing hypersensitivity.[4,5]

The accepted mechanism involved in the pathogenesis of DH is the hydrodynamic theory, developed in the 1960s by Brannstrom and co-workers.[2] According to this theory, the dentinal tubules when exposed to thermal, chemical, tactile, or evaporative stimuli, the fluid present within the tubules shows movement thereby stimulating mechanical receptors that are sensitive to fluid pressure. These mechanoreceptors transfer the stimuli to the pulpal nerve endings, eventually inducing pain response.[2]

The goal of treating dentinal hypersensitivity is to relieve the pain using a variety of agents such as potassium nitrate, strontium chloride hexahydrate, bioactive glass, calcium phosphate and glutaraldehyde to name a few. Recently, lasers, including the Erbium-YAG , the GaAlAs (Gallium-Aluminium-Arsenide), and the Neodymium: Yttrium Aluminium Garnet (Nd-YAG) have also been proven to be effective in treating DH.[6]

The possible mechanism by which various desensitizing dentifrice agents act is via precipitation, coagulation and melting of the dentin leading to occlusion of the dentinal tubules whereas laser irradiation causes photobiomodulatory effects leading to nerve depolarization of C-fibres hence, causing instant analgesia.

However, there is need for an agent that can provide immediate relief over pain and simultaneously remineralize the tooth.

One such agent is NovaMin®, i.e. calcium sodium phosphosilicate (CSPS) a synthetic mineral made of calcium, sodium, phosphorus and silica as well as other elements that releases deposits of crystalline hydroxyapatite, which is physically comparable to the mineral content the tooth.[8] It mainly acts through the production of bioglass, which reacts with saliva in the mouth to form a protective layer of hydroxyapatite on the tooth surface thus, occluding dentinal tubules.[15] Studies have also advocated that NovaMin® attaches itself to an exposed dentin surface and reacts to create a mineralized layer.[15] The layer is both mechanically robust and resistant to acid attacks. Additionally, there is a constant release of calcium over time, creating tubular occlusion all throughout.[16]

The use of lasers in treatment of dentinal hypersensitivity has gained more attention in recent times due to its property in creating analgesic effects within a short period of time with minimal adverse effects. Depending on the type of laser and the parameters used, the efficiency of different types of lasers: Nd-YAG, GaAlAs, and Erbium-YAG lasers for treating dentinal hypersensitivity varies from 5.2 to 100%. Although the exact mechanism of lasers in treating dentin hypersensitivity is unknown, it has been hypothesized that laser irradiation causes melting of the dentin surface through partial fusion and recrystallization of dentinal tubules components.[20] Matsumoto et al reviewed use of GaAlAs laser for the treatment of dentinal hypersensitivity to be highly beneficial.[17]

It has been ascertained through scanning electron microscope (SEM) analysis that the use of NovaMin® bioactive glass and lasers help in the formation of calcium hydroxyapatite layers and dentinal fluid coagulation, respectively, which help obliterate the dentinal tubule and decrease dentinal hypersensitivity.[12]

Thus, dentine discs invitro model given by Gilliam et al[27] would seem to be the approach of choice to understand the efficacy of various desensitizing agents because it is simple to use, reproducible and offers a flat surface for elemental analysis. Desensitizing agents and low power lasers can be applied with ease to the dentinal tubules when sectioned in a transverse plane. This makes the selection and assessment of appropriate treatment modalities a crucial element in experimental design.[27]

Therefore, the present study is undertaken with an aim to compare diode laser (940 nm) with a commercially available desensitizing agent and a combination of both on the occlusion of dentinal tubules.

AIM OF THE STUDY:

To assess and compare the effect of diode laser (940nm), commercially available toothpaste containing desensitizing agent and combination of diode laser (940nm) along with commercially available toothpaste containing desensitizing agent on dentinal tubule occlusion using scanning electron microscope.

OBJECTIVES OF THE STUDY:

1. To evaluate the efficacy of commercially available toothpaste containing desensitizing agent on the occlusion of dentinal tubules.
2. To evaluate the efficacy of diode laser (940nm) on the occlusion of dentinal tubules.
3. To evaluate the efficacy of diode laser (940nm) along with commercially available toothpaste containing desensitizing agent on the occlusion of dentinal tubules.
4. To compare the efficacy of diode laser (940nm), commercially available toothpaste containing desensitizing agent and combination of diode laser (940nm) along with commercially available toothpaste containing desensitizing agent on the occlusion of dentinal tubules.

REVIEW OF LITERATURE

- "Dentinal hypersensitivity" (DH) is the most common complaint amongst patients in dental practice. Incidence of "DH" 10% to 30%.[1] The possible etiology of this is because of wasting disease like attrition, abrasion, abfraction and erosion, gingival recession, pulpal damage etc.[2]
- Tailor-made treatment plan starts with most non-invasive treatment options followed by extensive procedures, in order to alleviate symptoms. Use of non-surgical and non-invasive methods such as use of desensitizing agents, laser therapy/ combinations should be considered as primary treatment option if the hypersensitivity doesn't subside than one should go for invasive treatment options, such as root-coverage etc.[3,4]

1.) **Surbhi P et al. (2019)[6]** reviewed that dentinal hypersensitivity a familiar and notable dental problem which may be defined - brief, sharp pain arising from exposed dentin. The primary cause of gingival recession is excessive tooth brushing, leading to tooth abrasion causing sensitivity of teeth due to dentin exposure. Most accepted theory for hypersensitivity "hydrodynamic theory by Brannstrom in 1964" which states: when the exposed dentin comes in contact with stimuli, it causes movement of fluid inside, excites pressure sensitive receptors leading painful response.

2.) **Bubteina N, et al (2015)[7]** stated in review: although "DH" condition that can occur at any age, incidence most commonly seen in the age range of 20-50 years. "DH" can be managed by very wide variety of procedures, agents, formulations applied locally, either "in office" or "at home". Dental practitioner should first identify causative/ predisposing factor after taking thorough history before treatment plan designed. Treatment plan of DH starts with selfcare management and later may be supplemented with professional interventions depending on severity of case.

3.) **Chun-Hung C et al. (2010)**[8] mentioned in review that DH occurs in reaction to chemical, thermal, evaporative or osmotic stimuli and cannot be attributed as any other dental defects or pathology. Hong Kong survey found prevalence of dentin hypersensitivity greater than 60% among patients attending dental hospital; lower incisors were most commonly affected teeth. Various authors have found that many dental clinicians have misconceptions about dentin hypersensitivity and hence lack confidence to manage for DH. When patient presents with signs that may be attributed to dentin hypersensitivity, thorough clinical evaluation must be carried out to rule out other likely causes before making diagnosis and starting on treatment.

4.) **Sanjay M et al. (2009)**[3] published a review stating that "DH": common clinical condition usually associated with exposed dentinal surfaces. It can affect patients of any age group and most commonly affects canines and premolars of both arches. Article concisely reviews the pathophysiology, mechanism and clinical management of the DH. Treatment of DH should start with precise diagnosis. There various treatment modalities available which can be used at home or may be professionally applied. Article also discusses the recent treatment options like bioglass, Portland cement, lasers and casein phosphopeptide.

5.) **Walters PA et al (2005)**[9] reviewed that pain response varies from one individual to another. DH involves facial surfaces of teeth near cervical aspect, very common in premolars, canines. Most widely accepted theory of how pain occurs is Brannstrom's hydrodynamic theory, fluid movement within dentinal tubules. Dental professional, using variety of diagnostic techniques, will discern condition from other conditions that may cause sensitive teeth. Treatment of condition can be invasive or non-invasive in nature. Most inexpensive, efficacious first line of treatment for most patients is dentifrice containing desensitizing active ingredient: potassium nitrate, stannous fluoride.

DESENSITIZING AGENT- "NovaMin® (calcium sodium phosphosilicate)"

- To understand science behind unique properties and reactivity of CSPS materials, its important to know how science developed for bone regenerative medicine translated directly to area of oral healthcare. "NovaMin®" branded ingredient found in number of professional use dental products designed to give immediate and long-lasting relief from tooth sensitivity.[9] "NovaMin®" technically described as inorganic amorphous calcium sodium phosphosilicate (CSPS) material designed based on class of materials known as bioactive glasses. Particular composition of NovaMin is identical to one the best-known bioactive glass material. NovaMin®" and other "CSPS" materials were originally developed as bone regenerative materials in the early 1970s. "CSPS" materials were part of a broader class of bioactive ceramics, which included calcium phosphate materials, calcium hydroxyapatite materials, developed for hard tissue repair and replacement, mainly due to their chemical similarity to bone mineral.[10]

6.) **Arantes et al (2019)[11]** conducted systematic review/ meta-analysis. Randomized, non-randomized clinical trials comparing DH reduction in adults given Pro-Argin-containing toothpastes “NovaMin®” containing toothpastes were included. No statistically significant difference between two toothpastes for DH reduction observed at immediate follow-up. Certainty of evidence: very low. Included studies presented high risk of bias. Concluded: pro-Argin-containing & NovaMin-containing toothpastes showed effectiveness for DH reduction. No statistically significant difference between two toothpastes was found.

7.) **Shah, S et al. (2017)**[12] did an SEM study to know the efficacy of NovaMin- and Pro-Argin-containing desensitizing dentifrices on occlusion of dentinal tubules. All three desensitizing dentifrices − SHY-NM, Sensitive Pro-Relief and Thermoseal – demonstrated varying degrees of tubular occlusion. New NovaMin-containing toothpaste −SHY-NM − resulted in better tubular occlusion, its use could be indicated for management of dentin hypersensitivity for obtaining more credible results

8.) **Teresa May Layer (2011)**[13] published review regarding development of fluoridated, daily-use toothpaste containing NovaMin® technology for the treatment of dentin hypersensitivity. Calcium sodium phosphosilicate (trade name NovaMin®) inorganic amorphous compound contains only “calcium, sodium, phosphate, and silica”. They concluded this product, based upon 5% w/w CSPS technology, has been shown to deliver a hydroxyapatite-like reparative layer to the surface of the dentin in vitro which is robust and resistant to acid challenge.

9.) **David C. Greenspan et al. (2010)**[14] did study on use of CSPS in periodontal surgery, where tooth sensitivity routinely found, coupled with need for improved materials to treat tooth sensitivity, led to initial investigations of this material for treating tooth sensitivity. NovaMin® had shown that these materials will actually form strong attraction with collagen. Because dentin consists of collagen to significant proportion, "NovaMin®" particles would bind to exposed dentin surface, physically fill open tubules. It further hypothesized that subsequent ionic release, surface reaction help form protective hydroxyl carbonate apatite layer that would impart rapid and continual relief from tooth sensitivity.

LOW LASER THERAPY

- Low level laser therapy to reduce pain, inflammation and edema, to promote wound, deeper tissues and nerves healing, and to prevent tissue damage has been known for almost forty years since the invention of lasers.[14] Mitochondria are thought to be a likely site for the initial effects of light, leading to increased ATP production, modulation of reactive oxygen species, and induction of transcription factors.[14]

10.) **McCarthy D et al (2020)[18]** conducted an in vitro pilot study was undertaken to investigate the effects of Neodymium: Yttrium Aluminium Garnet (Nd: YAG), Erbium: Yttrium Aluminium Garnet (Er:YAG) and Helium Neon (HeNe) laser radiation on dentine surfaces in extracted human teeth. Instrumented root surfaces and etched and unetched dentine discs were irradiated with a Nd:YAG laser operating at 3.5, 3.75 or 4W or an Er:YAG laser operating at 60, 80, 100mJ or with a HeNe laser. It was found Nd:YAG laser produced melting and resolidification of dentine on all surfaces while the Er:YAG laser produced craters with closed tubules in root surfaces, but open tubules in dentine discs. The HeNe laser produced no apparent surface alteration. Both lased and unlased control surfaces were compared using Scanning Electron Microscopy (SEM).. Nd:YAG radiation produced irregular melting and resolidification of the dentine surface with some occlusion of open dentine tubules. Er:YAG radiation caused ablation of dentine and produced craters with open tubules in the dentine discs, but closed tubules on most root surfaces. Irradiation using a HeNe laser produced no noticeable surface effect.

11.) **Roberto B et al. (2016)**[19] conducted a literature review to evaluate the effectiveness of the laser-assisted treatment of dentinal hypersensitivity. The review was performed from January 2009 to December 2014 with electronic data-bases: Medline via PubMed, Science Direct and Cochrane Library. Research of paper magazines by hand was not considered. Forty-three articles were selected between literature reviews, *in vitro* studies, clinical trials, pilot and preliminary studies. The items were divided into laser-used groups for an accurate description, and then the reading of results into various typologies. They concluded that Laser-assisted treatment reduces dentinal hypersensitivity-related pain, but also a psychosomatic component must be considered, so further studies and more suitable follow-ups are necessary.

12.) **Asnaashari M et al (2013)**[20] conducted a literature review on effectiveness of lasers in the treatment of dentin hypersensitivity which focuses on definition, diagnosis, etiology, predisposing factors, various laser types in the treatment of DH alone or in combination with topical desensitizing agents. Since a certain treatment has not yet been introduced for dentin hypersensitivity, a combination of laser therapy and topical desensitizing factors, can increase the success of the treatment compared with either treatments alone.

13.) **Ying Liu et al (2013)**[21] conducted an in vitro study to investigate the ultrastructural changes of dentin irradiated with 980-nm diode laser under different parameters and to observe the morphological alterations of odontoblasts and pulp tissue. 20 extracted human third molars were selected to prepare dentin discs. Each dentin disc was divided into four areas and was irradiated by 980-nm diode laser under different parameters. The result demonstrated that dentinal tubules can be entirely blocked after irradiation by 980-nm diode laser, regardless of the parameter setting. Diode laser with settings of 2.0 W and 980-nm sealed exposed dentin tubules effectively, and no significant morphological alterations of the pulp and odontoblasts were observed after irradiation. Irradiation with 980-nm diode laser could be effective for routine clinical treatment of DH, and 2.0W/CW (166 J/cm) was a suitable energy parameter due to its rapid sealing of the exposed dentin tubules and its safety to the odontoblasts and pulp tissue.

14.) **Gholami et al (2011)**[22] performed an invitro study where they evaluated the occluding effects of Er;Cr:YSGG, Nd:YAG, CO2 and diode lasers on dentinal tubules using a scanning electron microscope (SEM). Fifteen human third molars were collected and ground-sectioned vertically on the buccal and lingual surfaces to yield two dentin disks of 2-mm thickness. Four sites for laser irradiation and a control site were marked in the cervical areas of the dentin disks. Before laser application, specimens were exposed to a 14% EDTA solution and were then evaluated by SEM. The tubules entrance diameters were determined by ‘‘scale-bar’’ software which is specifically designed for SEM. The mean dentinal tubule entrance diameters for Er:Cr:YSGG, 810-nm diode, CO2, and Nd:YAG, were 1.73, 3.27, 2.10, and

1.64 microns, respectively, compared with 3.52 microns before laser irradiation. Overall, the furthest reduction in mean tubule diameter resulted from the Nd: YAG laser (53%). However, tubular diameter reduction in all laser groups ($p < 0.05$) was found to be statistically significant. They concluded that Nd: YAG, Er; Cr: YSGG, and CO2 lasers, through their ability to melt peritubular dentin, can occlude dentinal tubules partially or totally, and therefore reduce patients' hypersensitivity symptoms. The 810-nm diode laser sealed tubules to a far lesser degree, with negligible effects on desensitization.

15.) **Al-Saud LM et al (2003)**[23] conducted an in vitro study to microscopically evaluate and compare the occluding effect of the Nd: YAG laser and different dentin desensitizing agents on human dentinal tubules. Nd: YAG laser-irradiated dentin showed reduction or complete obliteration of the dentinal tubule lumen; thus, the treatment modified the original dentinal structure. The lased dentin surface in the two-minute group showed bubble-like changes in the area of the dentinal tubules' orifices. Statistically, the two-minute group was found to have a significantly higher percentage of partially or fully occluded tubules than did the one-minute group. All of the studied desensitizing agents produced occlusion of the dentinal tubules; however, the appearance of the precipitates, the level of coverage, and the degree of dentinal occlusion varied among the tested products. Throughout the specified period of this study, occlusion and/or narrowing of the open dentinal tubules have been successfully achieved with both treatment approaches.

16.) **Marsilio et al (2003)**[24] conducted a clinical study on the Clinical Application of the GaAlAs Laser in the Treatment of Dentine Hypersensitivity. This exposure can result after removal of the enamel and/or dental cement, or after root denudation. Different treatments are proposed for this disorder. 25 patients, with a total number of 106 cases of DH, were treated with GaAlAs low-level laser therapy (LLLT). 65% of the teeth were premolars; 14% were incisors and molars; 6.6% were canines. The teeth were irradiated with 3 and 5 J/cm2 for up to six sessions, with an interval of 72 h between each application, and they were evaluated initially, after each application, and at 15- and 60-days follow-up post-treatment. It was found that the treatment was effective in 86.53% and 88.88% of the irradiated teeth, respectively, with the minimum and maximum energy recommended by the manufacturer. There was a statistically significant difference between DH and after a follow-up of 60 days for both groups. The difference among the energy maximum and minimum was not significant. Hence, the GaAlAs low-level laser was effective in reducing initial DH. A significant difference was found between initial values of hypersensitivity and after 60 days follow-up post-treatment

<u>COMBINATION THERAPY</u>

17.) **Shetty M et al. (2020)**[25] conducted a comparative evaluation of novel desensitizing agents on Dentinal Tubule Occlusion. NovaMin (SHY NM) and laser diode therapy showed a greater number of completely occluded tubules and Gluma desensitizer and Colgate sensitive Pro-relief produced a greater number of partially occluded tubules. There was a statistically significant difference between the five groups when the ratio of complete and partial occlusion was calculated against the total number of tubules. Hence, the Diode Laser application and NovaMin (SHY NMTM) application could be more effective in providing relief from dentinal hypersensitivity.

18.) **Reddy G et al. (2017)**[26] did a comparative scanning electron microscope analysis of diode laser and desensitizing toothpastes for evaluation of efficacy of dentinal tubular occlusion. Diode laser (Group III) has shown more efficacy in occluding dentinal tubules when compared with desensitizing toothpastes, i.e., NovaMin (Group I) and Pro-Argin (Group II) which was statistically significant. The efficacy of diode laser when combined with the desensitizing toothpaste (Group IV – NovaMin +diode laser and Group V – Pro-Argin+ diode laser) was more when compared to Group III (diode laser) which was not statistically significant. Comparison of percentages of CO tubules among the five groups showed that Group IV (N + DL) showed the highest percentage of CO dentinal tubules.

CITRIC ACID CHALLENGE

- Often our oral cavity encounters low pH while we have citrus or sour food items and most individuals have dentinal hypersensitivity due to these food items. Hence to create same low pH environment the citric acid challenge is used in invitro dentinal disk model is order to see variability in dentinal tubular occlusion.

19.) **Pereira et al (2018)**[27] did a comparative evaluation of desensitizing dentifrices containing Biomin®, NovaMin® and fluoride on dentinal tubule occlusion before and after a citric acid challenge. Forty-five dentine specimens with patent tubules were randomly divided into 3 groups (n=15), Group A: brushing with Biomin; Group B: brushing with Novamin®, and control Group C: brushing with fluoride. The percentage of tubule occlusion (%OCT) of representative images from each group was analyzed using an environmental

SEM. The %OCT with BioMin® containing dentifrice was significantly higher than NovaMin® and a control i.e., fluoride containing dentifrice. Biomin ® and Novamin® containing dentifrices showed significant citric acid resistant compared to the fluoride containing dentifrice.

20.) **Sneha A et al. (2017)**[28] conducted the study to compare the effects of two desensitizing dentifrices containing NovaMin® and arginine on dentinal tubule occlusion with and without citric acid challenge in vitro using confocal laser scanning microscopy (CLSM). Forty dentin discs were randomly divided into Groups I and II containing twenty specimens each, treated with NovaMin and arginine-containing dentifrices, respectively. Groups I and II were divided into subgroups A and B where IA and IIA underwent CLSM analysis to determine the percentage of tubule occlusion while IB and IIB underwent 0.3% citric acid challenge and CLSM analysis. A novel grading system was devised to categorize tubule occlusion. In Group II, the percentage of occluded tubules was highest for IIA (72.25% ± 10.57%) and least for IIB (42.55% ± 8.65%) having statistical significance ($P < 0.0005$). In Group I, the difference between IA (49.9% ± 12.96%) and IB (43.15% ± 12.43%) was statistically insignificant ($P = 0.249$). On the comparison between IB and IIB statistically indifferent result was obtained ($P = 0.901$), whereas the difference between IA and IIA was statistically significant ($P < 0.001$). The results of grading system were for IA 50% of samples belonged to Grade 2, for IIA 60% - Grade 3, and for IB 70% and for IIB 90% - Grade 2. Hence, dentinal tubule occlusion with arginine-containing dentifrice was significantly higher than NovaMin®. However, it could not resist citric acid challenge as effectively as NovaMin®.

The effects of NovaMin® were more sustainable as compared to arginine-containing dentifrice, thus proving to be a better desensitizing agent.

21.) **Wang Z et al. (2010)**[29] carried out this study to evaluate the in vitro effectiveness of a new bioglass-containing and two commercial desensitizing toothpastes on dentinal tubule occlusion after citric acid challenge or artificial saliva (AS) immersion. One hundred dentin discs from human third molars were used. Specimens were randomly divided into five groups (n = 20), Group 1: EDTA-treated dentin; Group 2: brushing with distilled water; Group 3: brushing with Novamin; Group 4: brushing with Sensodyne Fresh mint; Group 5: brushing with Colgate Sensitive. In each group, samples were then equally split into two subgroups (n = 10) to test two post-treatments: 6% citric acid challenge or 24 h immersion in artificial saliva. Dentine permeability of each specimen was measured before and after each treatment using a hydrostatic device working at 20 cm H2O pressure. Data were analysed by two-way repeated measures ANOVA to determine if there were any significant differences within or between groups. Dentine morphology and surface deposits were observed by SEM. All three desensitizing toothpastes significantly reduced dentine permeability and created precipitates on the treated dentine surfaces. Moreover, the reductions in dentine permeability showed partial recovery after a citric acid and artificial saliva immersion. Sensodyne showed significant resistant to acid attack and Novamin exhibited the lowest permeability after artificial saliva immersion for 24 h. Hence it showed that the application of the three toothpastes resulted in effective dentinal tubule occlusion. However, the new bioglass-containing toothpaste (Novamin®) represented excellent occlusion effects after brushing treatment and AS immersion, while Sensodyne demonstrated more reduction in permeability when citric acid challenged.

SCANNING ELECTRON MICROSCOPE (SEM)[30,31]

- The Scanning Electron Microscope (SEM) is a microscope that uses electrons rather than light to form an image. There are many advantages for using the SEM instead of an optical microscope. [FIGURE-10]. The SEM has a large depth of field, which allows a large amount of the sample to be in focus at one time. The SEM also produces images of high resolution, which means that closely spaced features can be examined at a high magnification.[27] Preparation of the samples is relatively easy since most SEMs only require that sample should be conductive. The combination of higher magnification, larger depth of focus, greater resolution, and ease of sample observation makes the SEM one of the most heavily used instruments in current research and development. The electron beam comes from a filament, made of various types of materials. The most common is the tungsten hairpin gun. This filament is a loop of tungsten that functions as the cathode. A voltage is applied to the loop, causing it to heat up. The anode, which is positive with respect to the filament, forms powerful attractive forces for electrons. This causes electrons to accelerate toward the anode.[31] The anode is arranged, as an orifice through which electrons would pass down to the column where the sample is held. Other examples of filaments are Lanthanum Hexaboride filaments and field emission guns. The streams of electrons that are attracted through the anode are made to pass through a condenser lens, and are focused to very fine point on the sample by the objective lens (FIGURE-9). The electron beam hits the sample, producing secondary electrons from the sample. These electrons are collected by a secondary detector or a backscatter detector, converted to a voltage, and amplified. The amplified voltage is applied to the grid of the CRT that causes the intensity of the spot of light to change.[32]The image consists of thousands of spots of varying intensity on the face of a CRT that correspond to the topography of the sample. SEM is used for studying the surface topography, microstructure, and chemistry of metallic and non-metallic specimens at magnifications from 50 up to ~ 100, 000 X, with a resolution limit < 10nm (down to ~ 1nm) and a depth of focus up to several μm (at magnifications ~ 10,000 X). In SEM, a specimen is irradiated by an electron beam and data on the specimen are delivered by secondary electrons coming from the surface layer of thickness ~5nm and by backscattered electrons emitted from the volume of linear size ~ 0.5μm.[32]

MATERIALS AND METHODS

The present in vitro study was a comparative assessment of Diode Laser (940 nm), a commercially available Toothpaste containing desensitizing agent and a combination of both on the occlusion of dentinal tubules using scanning electron microscope – An in-vitro study.

This study was conducted in the Department of Periodontics KAHER's KLE Vishwanath Katti Institute of Dental sciences.

SOURCE OF DATA:

1. The study was conducted at "Department of Periodontics KAHER'S KLE V K Institute of Dental Sciences)" Belagavi Karnataka.
2. Healthy permanent teeth indicated for extraction were collected from 'Department of Oral & Maxillofacial surgery', KAHER'S KLE V K Institute of Dental Sciences' Belagavi Karnataka.
3. Artificial saliva was prepared in the 'Department of Biochemistry' 'KAHER'S Jawaharlal Nehru Medical College Belagavi' Karnataka.
4. Scanning Electron Microscope facility at Council of Scientific & Industrial Research-National Physical Laboratory- PUSA, New Delhi was used for analysis of the samples.

SAMPLE SIZE ESTIMATION

120 dentin discs of 5 mm diameter and thickness of 2 mm were obtained from healthy permanent extracted teeth [FIGURE-7].

Sample size was calculated by using a formula:

$$N = \frac{2s^2[Z\alpha+Z\beta]^2}{d2} = 30 = 30*4 = 120$$

$Z\alpha = 2.58$ at 1% α-error

$Z\beta = 1.682$ at 5% β-error

d = Absolute or margin of error

= 29.560

INCLUSION CRITERIA

120 well-formed intact human permanent teeth of systemically healthy individuals in the age group of 16-60 years indicated for extraction were collected for the study.

EXCLUSION CRITERIA

- Permanent teeth with dental caries.
- Permanent teeth with restoration.
- Permanent teeth with wasting diseases.
- Permanent teeth with developmental anomaly.

STUDY DESIGN:

The following study was an In-vitro study.

ARMAMENTARIUM USED IN STUDY

1. NovaMin® containing toothpaste (SHY-NM)
2. Diode Laser (BIOLASE- EPIC -X)→940nm
3. Toothbrush
4. EDTA- 0.5M

5. Normal saline
6. Citric acid→pH- 3
7. Artificial saliva involving various chemical agent
8. Scanning Electron Microscope
9. Slow speed water cooled handpiece

METHODOLOGY

ARTIFICIAL SALIVA PREPARARTION

Composition of the solution was $CaCl_2$= 11.6mg/100ml KCl =0.37gm/100ml KH_2PO_4= 78.9/100ml TRIS (buffer)= 0.302gms/100ml. The pH was adjusted to 7.4 (neutral PH). [FIGURE-4]

DENTIN DISC PREPARATION

120 healthy teeth extracted were stored in normal saline for a duration of one month, the teeth were then debrided thoroughly with the help of scalers & curettes post debridement. 120 dentin discs of 5 mm diameter and a thickness of 2 mm were obtained from diamond discs mounted on a slow speed water cooled handpiece [FIGURE-5]. Surface of each dentin disc was polished with 600 grit silicon carbide paper for 30seconds using back & forth motion. Smear layer was removed by immersing all dentin discs in 0.5M EDTA solution in a petri dish for 2 minutes. Discs were removed from EDTA & immersed in deionized water for 30 seconds [FIGURE-6][FIGURE-7].

DIVISION OF GROUPS

GROUP-1[C]-CONTROL (N=30)- Dentin discs treated with distilled water.

GROUP-2[T]-TOOTHPASTE (N=30)- Dentin discs treated with NovaMin® containing toothpaste (SHY-NM) [FIGURE-8].

PROCEDURE FOR TOOTHPASTE APPLICATION

Brushing with NovaMin® containing toothpaste (SHY-NM) was carried out for 2 mins on both front and back surface of each dentin disc using kid's toothbrush with small head & medium bristles followed by water rinse. It was kept in artificial saliva and sent for SEM analysis.

GROUP-3[L]-LASER (N= 30)→ Biolase epic-X-diode laser (940 nm) irradiation [FIGURE-8].

PROCEDURE FOR LASER APPLICATION

BIOLASE EPIC-X 940nm laser device with power setting 1W, and pulse interval of 200µm was applied in non contact mode for 60 seconds on both front and back surface of the dentin disc followed by water rinse. It was kept in artificial saliva and sent for SEM analysis.

GROUP-4[T+L]-COMBINATION OF TOOTHPASTE AND LASER (N=30)→ NovaMin® toothpaste + Biolase epic X diode laser (940 nm) irradiation [FIGURE-8].

PROCEDURE FOR APPLICATION OF TOOTHPASTE AND LASER IN COMBINATION

Brushing was carried out for 2 minutes on both front and back surface of each dentin disc using kids' toothbrush with small head & medium bristles followed by irradiation using BIOLASE EPIC-X 940nm laser surface with 1 W power, and pulse interval of 200μm in noncontact mode for 60 seconds on both front and back surface of the dentin disc followed by water rinse. It was kept in artificial saliva and sent for SEM analysis and data was obtained.

CITRIC ACID CHALLENGE

After SEM analysis dentin disc from **GROUP-1[C], GROUP-2[T], GROUP-3[L] and GROUP-4[T+L]** were immersed in a petri dish subjected to 0.3% citric acid with a sodium hydroxide buffer (NaOH) with pH of 3.2 for 5 minutes [FIGURE-3].The groups of dentin discs were labelled as **SUBGROUP-1[C], SUBGROUP-2[T], SUBGROUP-3[L] and SUBGROUP-4[T+L]** respectively.

SCANNING ELECTRON MICROSCOPE ANALYSIS:

Dentin discs were rinsed under distilled water & dried. Gold sputtering was done. The photomicrographs of surface from the centre of each dentin disc was obtained using FESEM Zeiss EVO MA 10, Oxford INCA 250 [FIGURE-9] [FIGURE-10].

PHOTOGRAPH MAGNIFICATION →The percentage of occluded tubules for each photomicrograph of dentin disc was calculated using the formula below. The results were calculated post treatment [Table1] [GRAPH1] and post citric acid challenge [Table 5] [GRAPH 3]

$$\frac{\text{Number of occluded tubule}}{\text{Total number of tubules}} \times 100$$

ARMAMENTERIUM

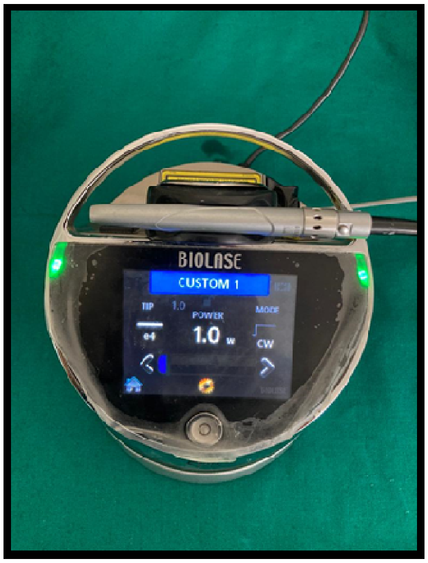


FIGURE-1 DIODE LASER BIOLASE EPIC X 940 nm

FIGURE-2 NOVAMIN®CONTAINING TOOTHPASTE- SHY-NM

FIGURE-3 AGENTS: ETHYLENE DIAMINE TETRAACETIC ACID- 0.5 M, CITRIC ACID 50% SOLUTION

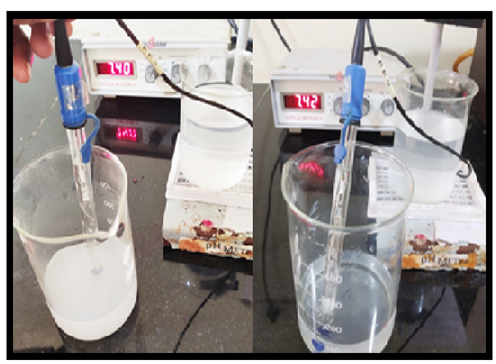


FIGURE-4 PREPARATION OF ARTIFICIAL SALIVA

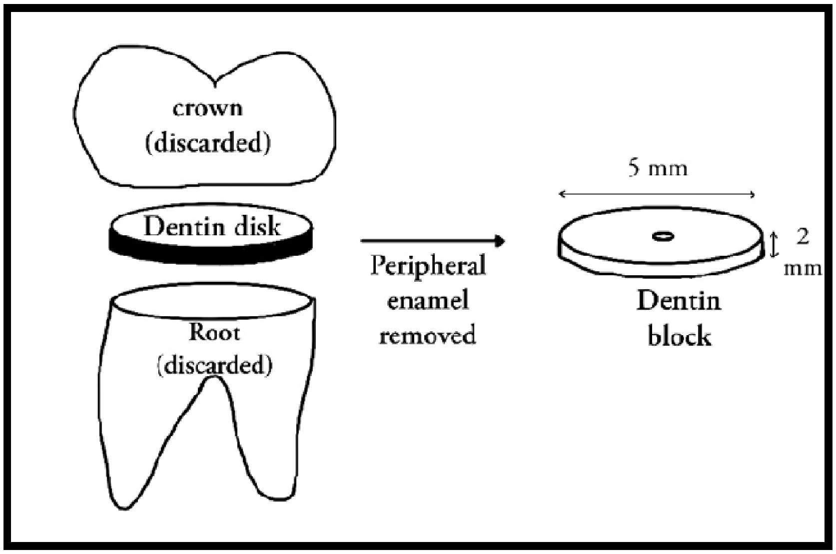


FIGURE-5 SCHEMATIC PRESENTATION OF DENTIN DISC

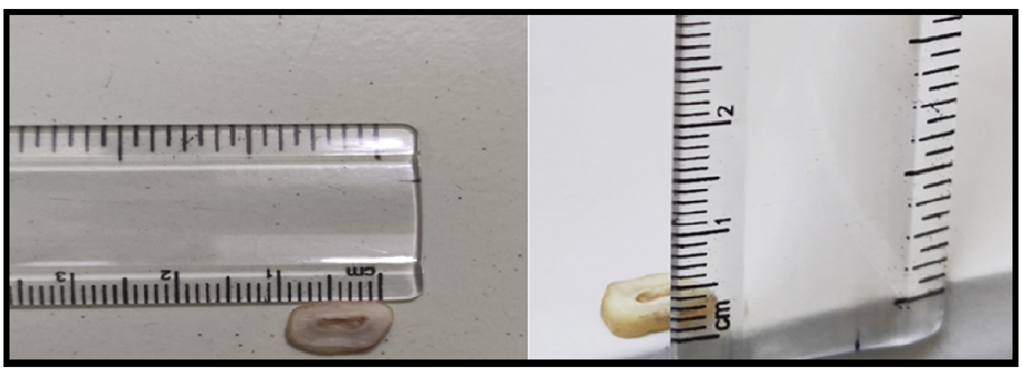


FIGURE-6 PREPARTION OF DENTIN DISC

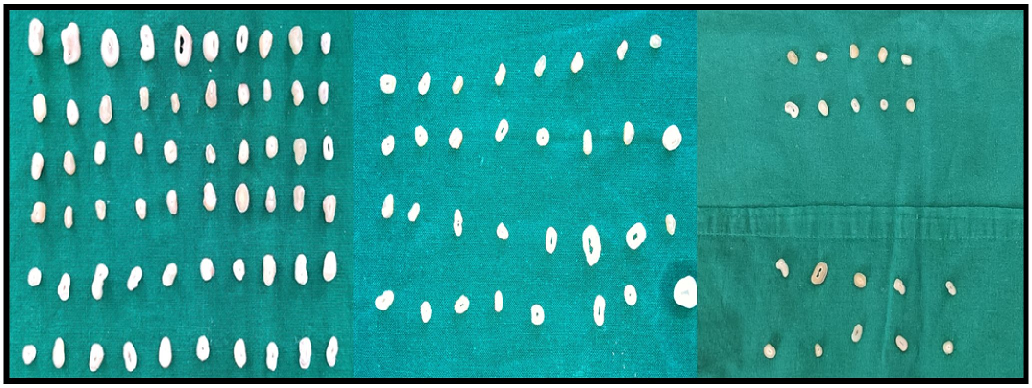


FIGURE-7 120 DENTIN DISCS

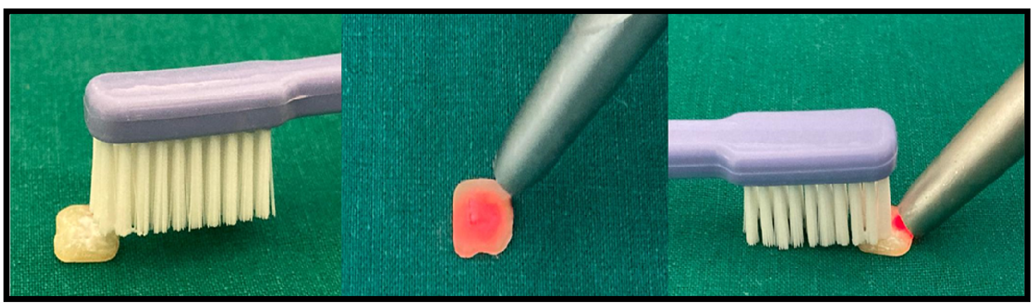


FIGURE-8 **GROUP 2 (T)** **TOOTHPASTE**	**FIGURE-8** **GROUP 3 (L)** **LASER**	**FIGURE-8** **GROUP 4 (T+L)** **TOOTHPASTE WITH LASER**

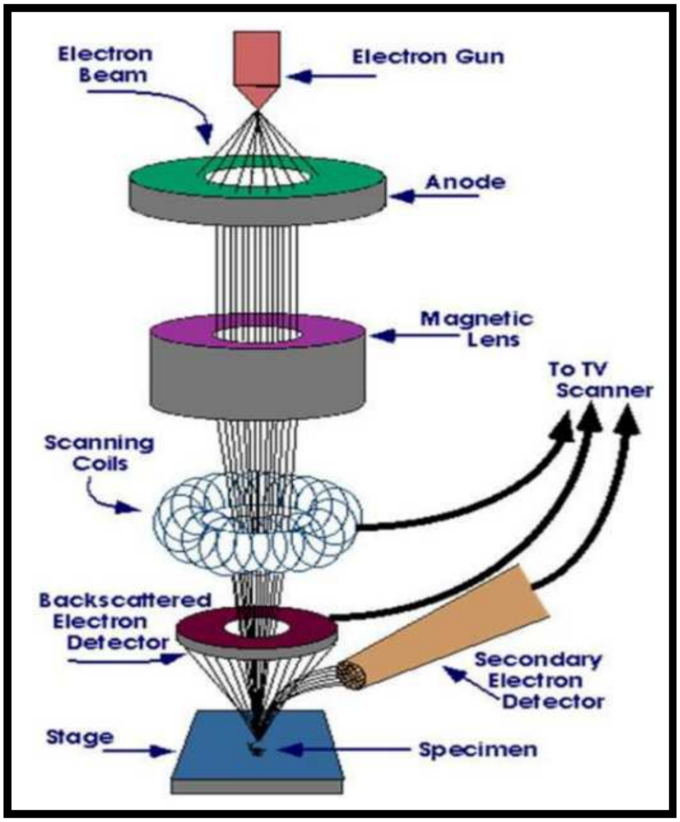


FIGURE-9 SCHEMATIC PRESENTATION [30,31] SCANNING ELECTRON MICROSCOPE

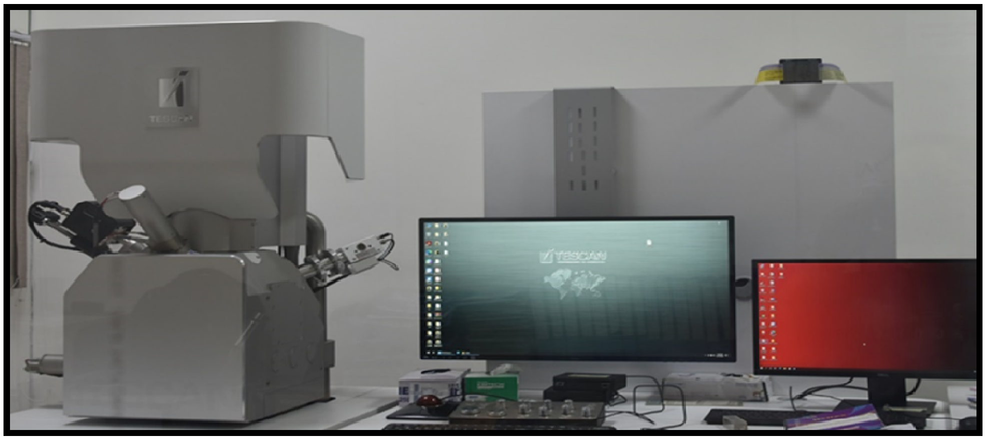


FIGURE-10 SCANNING ELECTRON MICROSCOPE- FESEM Zeiss EVO MA 10, Oxford INCA 250.

SCANNING ELECTRON MICROSCOPE PHOTO MICROGRAPHS BEFORE AND AFTER TREATMENT MAGNIFICATION- 5.00 kx, 10 µm

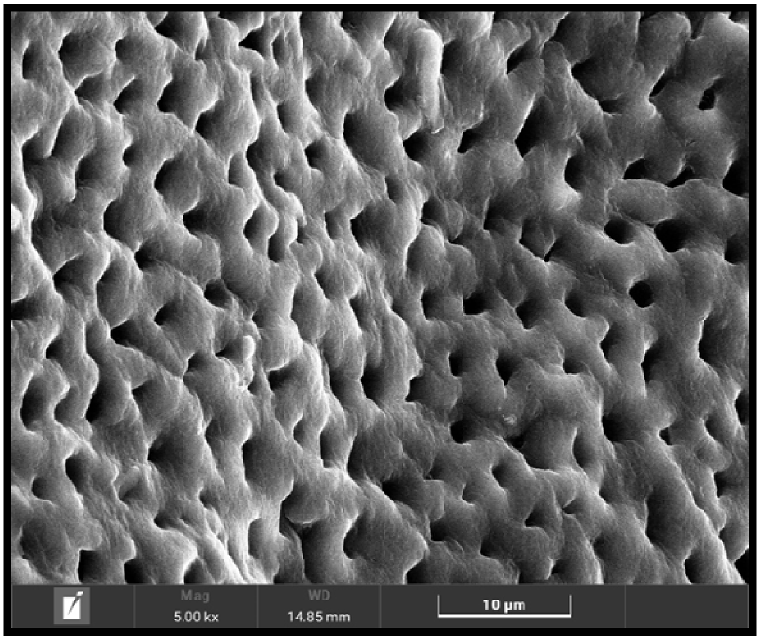


FIGURE-11 SEM PHOTOMICROGRAPH GROUP 1[C]- CONTROL- DISTILLED WATER

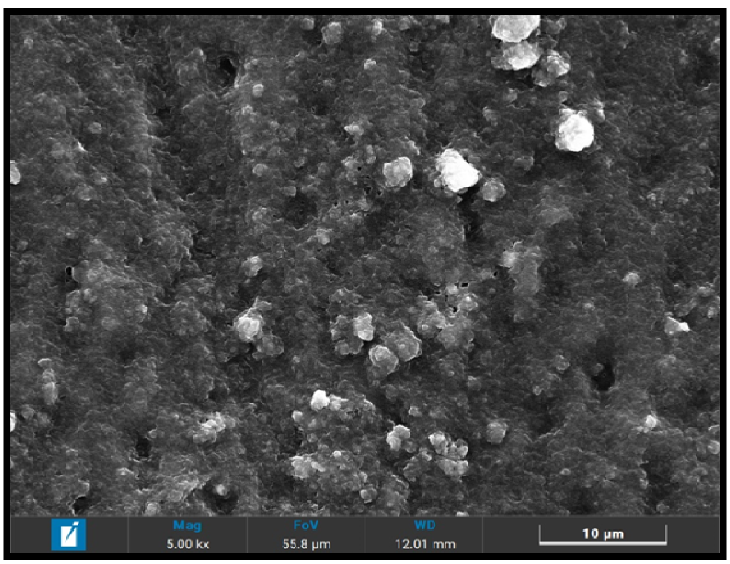


FIGURE-12 SEM PHOTOMICROGRAPH GROUP 2[T]- TOOTHPASTE- NOVAMIN® CONTAINING TOOTHPASTE (SHY-NM)

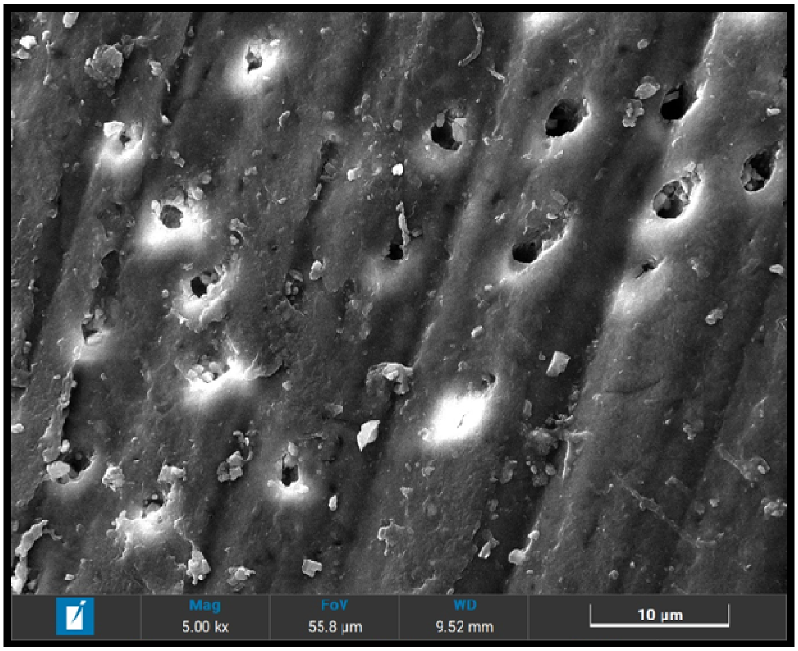


FIGURE-13 SEM PHOTOMICROGRAPH GROUP 3[L]-LASER - DIODE LASER- BIOLASE EPIC X 940NM

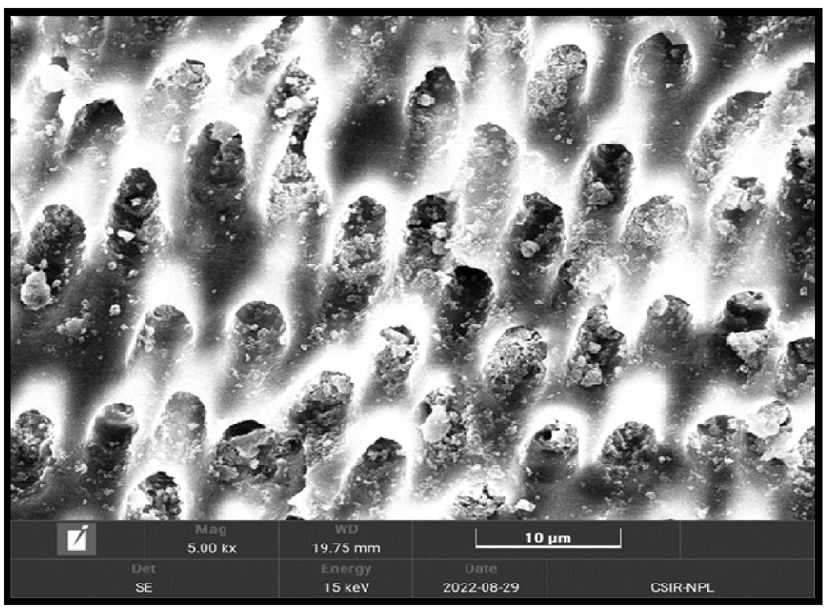


FIGURE-14 SEM PHOTOMICROGRAPH GROUP 4[T+L]-NOVAMIN® CONTAINING TOOTHPASTE (SHY-NM) + DIODE LASER- BIOLASE EPIC X 940NM

SCANNING ELECTRON MICROSCOPE PHOTO MICROGRAPHS POST CITRIC ACID CHALLENGE MAGNIFICATION- 5.00 kx, 10 µm

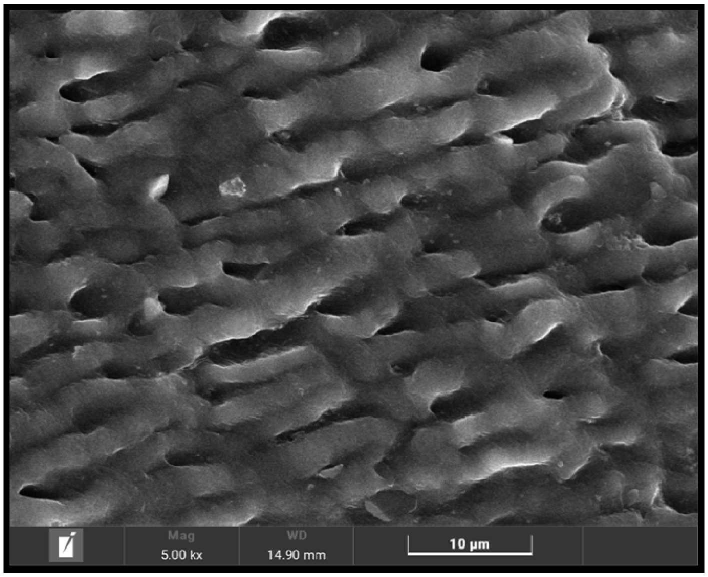


FIGURE-15 SEM PHOTOMICROGRAPH SUBGROUP 1[C]-CONTROL-DISTILLED WATER-POST CITRIC ACID CHALLENGE

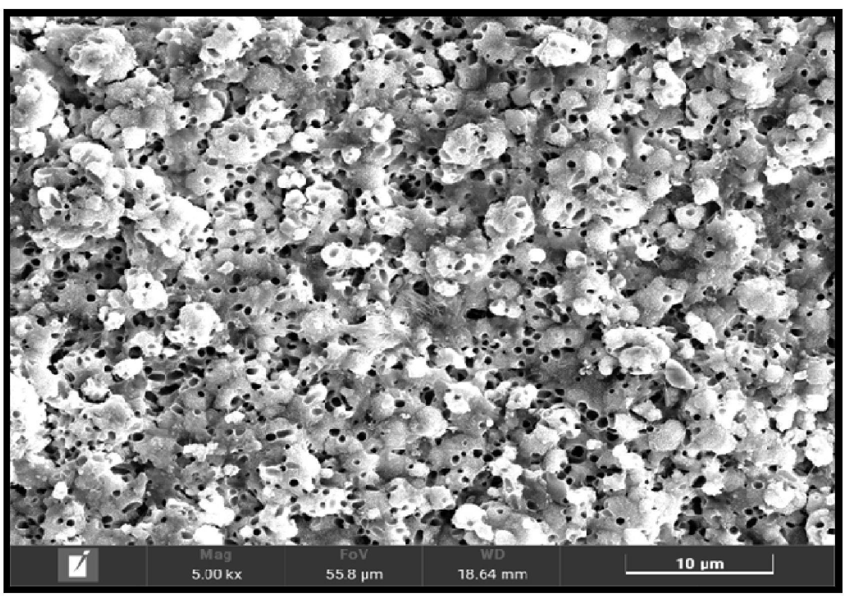


FIGURE-16 SEM PHOTOMICROGRAPH SUBGROUP 2[T]-NOVAMIN® CONTAINING TOOTHPASTE (SHY-NM)-POST CITRIC ACID CHALLENGE

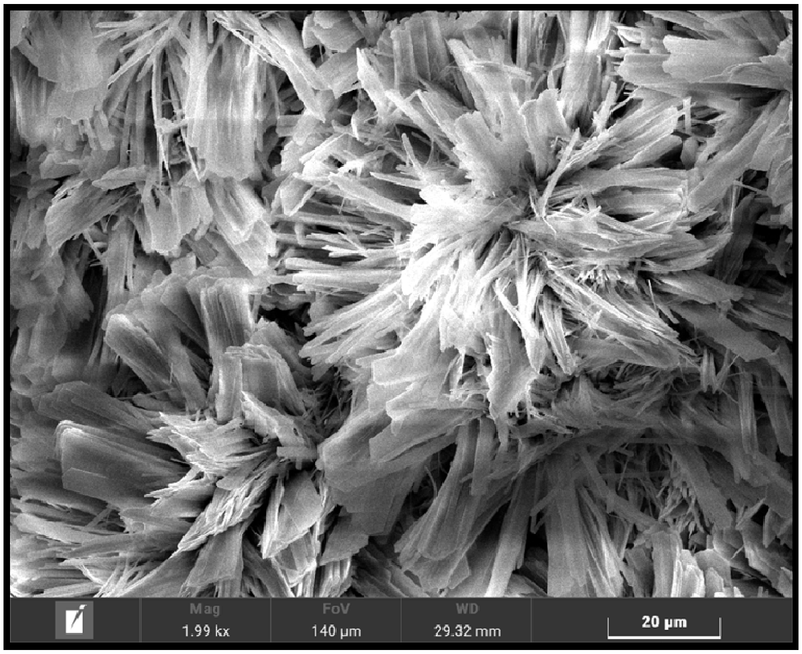


FIGURE-17 SEM PHOTOMICROGRAPH SUBGROUP 3[L]- LASER - DIODE LASER- BIOLASE EPIC X 940NM POST CITRIC ACID CHALLENGE

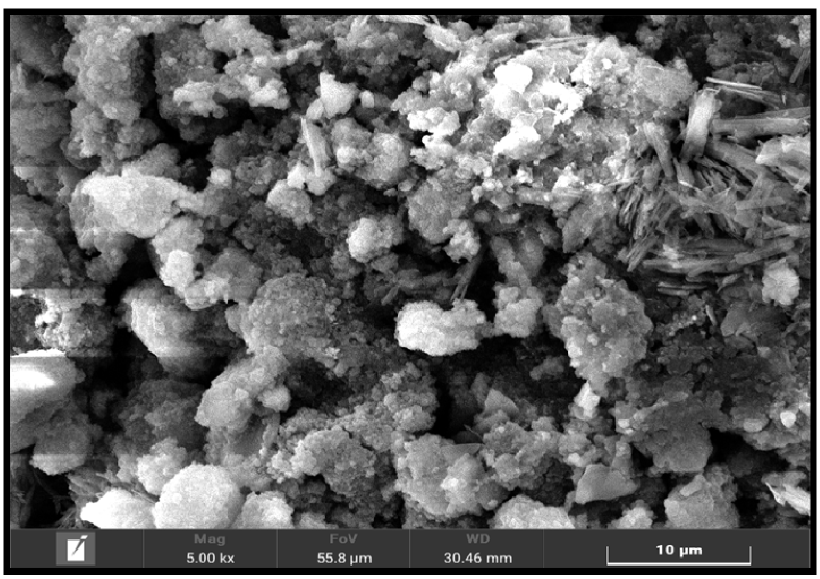


FIGURE-18 SEM PHOTOMICROGRAPH SUBGROUP 4[T+L]- NOVAMIN® CONTAINING TOOTHPASTE (SHY-NM) + DIODE LASER- BIOLASE EPIC X 940NM POSTCITRIC ACID CHALLENGE

RESULTS

STASTICAL ANALYSIS

The data were entered in Microsoft Excel and analyzed statistically using the SPSS software, version 21; SPSS Inc., (Chicago, IL, USA). The normality of the data was assessed prior to analysis using the Shapiro-Wilk's test/Kolmogorov-Smirnov test. Data were found to be normally distributed. Thus, parametric test was chosen. Descriptive analysis was calculated. Inter group comparisons was done by One way ANOVA. Pairwise Comparison of four groups was also carried out using post-hoc Tukey HSD Test. All statistical tests were performed at a significance level of 1% ($p<0.01$).

RESULTS AND OBSERVATION

In this study, 120 freshly extracted teeth, free from caries or any previous conservative or endodontic treatment, were used out of which 120 dentinal disks were prepared. The disks were divided into four groups having 30 dentinal disks in each group→

Group-1[Control][C]- Distilled water

Group-2[Toothpaste][T]- Novamin® toothpaste

Group-3[Laser][L]- Biolase epic-x diode laser (940 nm)

Group-4[Toothpaste + Laser] [T+L]- Combination of Novamin® toothpaste and of biolase epic-x diode laser (940 nm)

Followed by which scanning electron microscopy was done.

The data were entered in Microsoft excel and subjected to statistical analysis using SPSS software version 20.0.

The data were analysed for the percentage of dentinal tubular occlusion in all 120 dentinal disks. [TABLE 1] [GRAPH 1]. Further descriptive analysis was done for all groups. [TABLE 2] [GRAPH 2]. The data were statistically analysed with one-way analysis of variance (ANOVA) followed by Tukey's multiple post hoc test for pairwise comparison of the four groups with respect to the percentage of occluded tubules ($P < 0.01$). [TABLE 3 and TABLE 4]

Similarly, same specimens from groups 1[C],2[T], 3[L], and 4[T+L] went under citric acid challenge and were sub grouped →

Subgroup 1 [Control][C]- Distilled water post citric acid challenge

Subgroup-2 [Toothpaste][T]- Novamin® toothpaste post citric acid challenge

Subgroup-3 [Laser][L]- Biolase epic-x diode laser (940 nm) post citric acid challenge

Subgroup-4[Toothpaste +Laser] [T+L]- Combination of Novamin® toothpaste and of Biolase epic-x diode laser (940 nm) post citric acid challenge.

Followed by which scanning electron microscopy was done.

Again, data were entered in Microsoft excel and subjected to statistical analysis using SPSS software version 20.0.

The data were analysed for the percentage of dentinal tubular occlusion in all 120 dentinal disks post citric acid challenge. [TABLE 5] [GRAPH 3]. Further descriptive analysis was done for all subgroups. [TABLE 6] [GRAPH 4]. The data were statistically analysed with one-way analysis of variance (ANOVA) followed by Tukey's multiple post hoc test for pairwise comparison of the four subgroups with respect to the percentage of occluded tubules post citric acid challenge ($P < 0.01$) [TABLE 7 and TABLE 8].

TABLE 1: Percentage of dentinal tubular occlusion in all control and test groups.

GROUP-1[C] CONTROL (30) %	GROUP-2 [T] TOOTHPASTE (30) %	GROUP-3 [L] LASER (BIOLASE) (30) %	GROUP-4 [T+L] COMBINATION (30) (LASET+TOOTHPASTE) %
3	78	92	100
4	88	93	98
9	88	99	100
6	91	98	99
9	97	95	100
5	94	91	96
0	100	94	98
1	81	96	100
2	89	100	91
6	92	88	100
4	100	90	95
9	98	94	98
3	85	79	96
2	97	78	100
1	95	98	92
8	93	89	98
3	96	89	100
6	89	93	97
4	80	98	92
2	89	96	98
3	98	98	100
0	96	97	97
2	84	91	100
3	84	98	96
0	93	96	100
2	94	89	99
7	92	96	90
6	93	94	93
0	89	93	100
0	91	91	100

TABLE 2: Descriptive Analysis of dentinal tubular occlusion percentage of all groups.

C: Control; T: TOOTHPASTE L: Laser; T+L: Toothpaste + Laser

GROUPS	MEAN	STANDARD DEVIATION	STANDARD ERROR	p-VALUE
GROUP-1 [C] CONTROL	3.7000	2.8180	0.5145	Insignificant
GROUP-2[T] TOOTHPASTE	91.1333	5.8353	0.5835	*<0.001* (HS)
GROUP-3 [L] LASER	93.1000	5.2018	0.5365	*<0.001 (HS)
GROUP-4 [T+L] COMBINATION	97.7000	2.9261	0.2955	*<0.001 (HS)

*p value set at <0.01 it shows highly statistically significant (S) result at 99% Confidence Interval.

OBSERVATIONS:

1. Mean percentage and standard deviation of occluded dentinal tubules in treatment Group-2[T] was 91.13±5.8, for Group-3[L] was 93.1±5.2 and for Group-4[T+L] was 97.7±2.9.
2. Compared to group 2[T] and group 3[L] the percentage of occluded dentinal tubules was higher in group 4[T+L] that is 97.2%.
3. The three test groups are statistically significant as the p-value was found to be <0.001.

TABLE 3: Comparison of dentinal tubules occlusion in control and test groups using One-way ANOVA

ANOVA					
SOURCES	**Sum of Squares**	**Degree of freedom (df)**	**Mean Square (MS)**	**F statistics**	**p-value**
INTERGROUP	184,058.2250	3	61,352.7417	3,161.9973	<0.001
INTRAGROUP	2,250.7667	116	19.4032		

OBSERVATIONS:

1. One-way ANOVA was applied for comparison inter and intra groups.
2. The mean square value inter groups was 61,352.7417 and intra groups was 19.4032.
3. The difference inter and intra the groups was statistically significant with p-value<0.01.

TABLE 4: Intergroup and intragroup comparisons for control and test groups. (Multiple comparisons).

Tukey HSD Treatments Pair (GROUPS)	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inference
1[C] vs 2[T]	108.7180	0.0002	** p<0.01
1[C] vs 3[L]	111.1634	0.0002	** p<0.01
1[C] vs 4[T+L]	116.8832	0.0002	** p<0.01
2[T] vs 3[L]	2.4454	0.3135	insignificant
2[T] vs 4[T+L]	8.1652	0.0002	** p<0.01
3[L] vs 4[T+L]	5.7198	0.0002	** p<0.01

**p value set at <0.01 it shows significant (S) result at 99% Confidence Interval.

OBSERVATIONS:

1. Tukey's HSD test to each of the 6 pairs to pinpoint which of them exhibits statistically significant difference p<0.01.
2. On comparison, statistically significant differences were seen inter group 2[T] and group 4[T+L], group 3[L] and group 4[T+L] p=0.0002 respectively which was less than p-value<0.01indicating statistically significant results.
3. p-value for group 2[T] and group3[L] was p=0.313 which was greater than 0.01. Hence its statistically insignificant.

TABLE 5: Percentage of dentinal tubular occlusion in all control and test subgroups post citric acid challenge.

SUBGROUP-1 [C] CONTROL (30) %	SUBGROUP-2[T] TOOTHPASTE (30) %	SUBGROUP-3 [L] LASER (30) %	SUBGROUP-4[T+L] TOOTHPASTE+LASER (30) %
3	68	89	89
4	70	85	81
9	73	83	92
6	78	81	82
9	73	88	84
5	71	83	78
0	83	82	82
1	66	83	87
2	63	93	79
6	88	76	86
4	79	79	78
9	69	84	91
3	78	71	83
2	59	69	79
1	72	87	70
8	70	73	89
3	68	77	82
6	78	81	86
4	68	87	79
2	73	84	88
3	75	81	92
0	78	84	89
2	58	79	83
3	57	82	91
0	83	89	78
2	76	78	82
7	83	81	74
6	82	79	90
0	74	82	94
0	83	85	87

TABLE 6: Descriptive Analysis of dentinal tubular occlusion percentage of all subgroups post citric acid challenge. C: Control; T: TOOTHPASTE L: Laser; T+L: Toothpaste + Laser

GROUPS	MEAN	STANDARD DEVIATION	STANDARD ERROR	p-VALUE
CONTROL[C]	3.7000	2.8180	0.5145	*<0.001 (S)
TOOTHPASTE[T]	73.22	7.86699785	0.933640875	*<0.001 (S)
LASER[L]	81.83333333	5.305061561	0.582306156	*<0.001 (S)
COMBINATION[T+L]	88.26666667	4.841867218	0.510377618	*<0.001 (S)

*p-value set at <0.01 it shows statistically significant (S) result at 99% Confidence Interval.

OBSERVATIONS:

1. Mean percentage and standard deviation of occluded dentinal tubules in treatment subgroup-2[T] was 73.2±7.8, for subgroup-3[L] was 81.8±5.3 and for subgroup-4[T+L] was 88.2±4.8. p-value was found to be <0.001 indicating its statistically significant.

TABLE 7: Comparison of dentinal tubules occlusion in control and test subgroups post citric acid challenge using One-way ANOVA

ANOVA

SOURCES	Sum of Squares	Degree of freedom (df)	Mean Square (MS)	F statistics	p-value
INTER SUBGROUP	138,221.3667	3	46,073.7889	1,517.8521	<0.001
INTRA SUBGROUP	3,521.1333	116	30.3546		

OBSERVATIONS:

1. One-way ANOVA was applied for comparison inter and intra subgroups.
2. The mean square value inter subgroups was 46,073.7889 and intra subgroups was 30.3546.
3. The difference inter and intra subgroups was statistically significant with p-value<0.01.

TABLE 8: Inter subgroup and Intra subgroup comparisons of control and test subgroups post citric challenge (multiple comparisons).

Tukey HSD

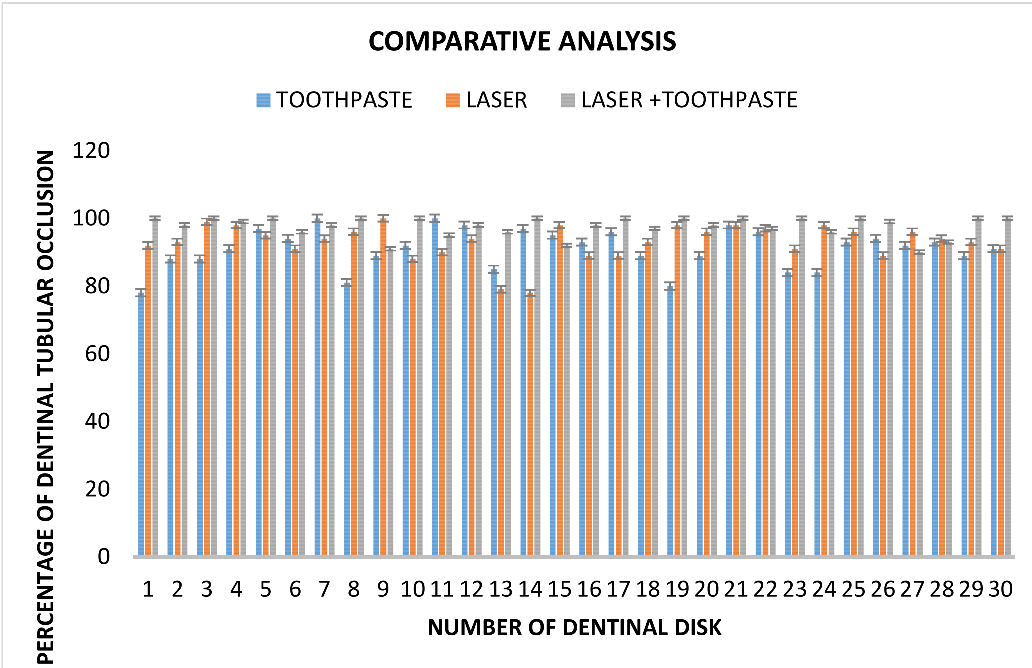
Treatments Pair (SUBGROUPS)	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inference
1[C] vs 2[T]	69.0929	0.0002	** p<0.01
1[C] vs 3[L]	77.6756	0.0002	** p<0.01
1[C] vs 4[T+L]	84.0713	0.0002	** p<0.01
2[T] vs 3[L]	8.5828	0.0002	** p<0.01
2[T] vs 4[T+L]	14.9784	0.0002	** p<0.01
3[L] vs 4[T+L]	6.3956	0.0002	** p<0.01

**p value set at <0.01 it shows significant (S) result at 99% Confidence Interval.

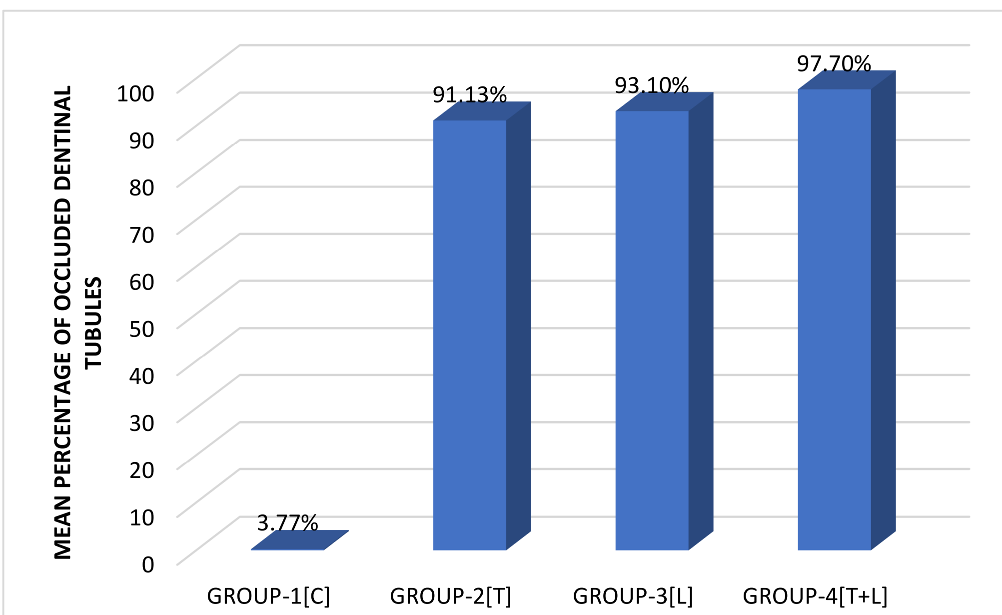
OBSERVATIONS:

1. Tukey's HSD test to each of the 6 pairs to pinpoint which of them exhibits statistically significant difference p<0.01.
2. On comparison, statistically significant differences were seen inter subgroup 2[T] and sub group 4[T+L], subgroup 2[T] and subgroup3[L], subgroup 3[L] and subgroup 4[T+L]
3. p-value=0.0002 in all the test subgroups indicating statistically significant result in all the test subgroups.

GRAPH-1- Comparative analysis of percentage of occluded dentinal tubular occlusion in all three test groups.

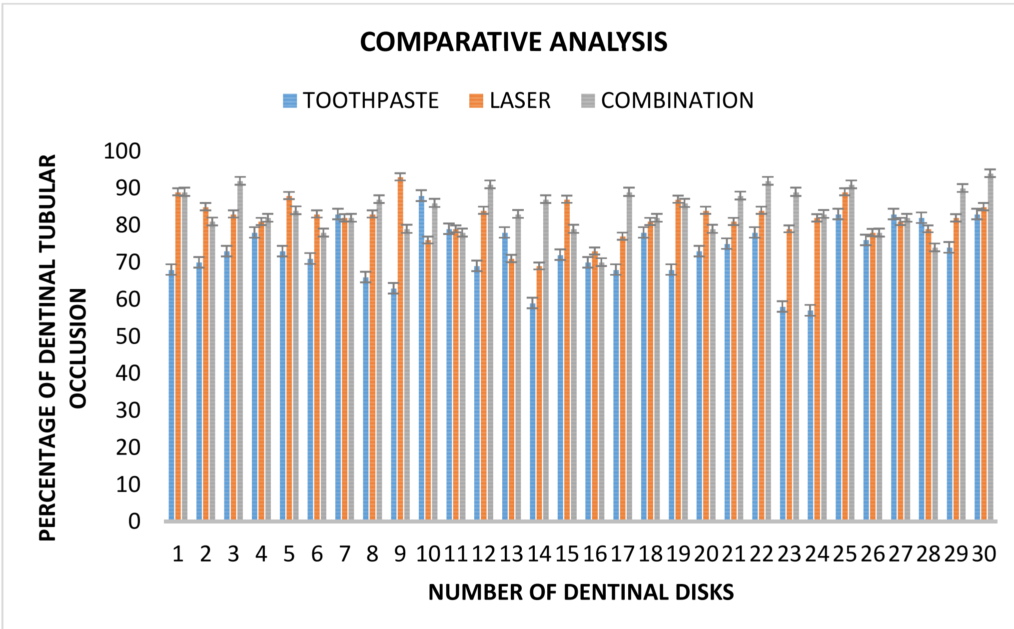


GRAPH-2-Mean percentage of occluded dentinal tubules in control and test groups.

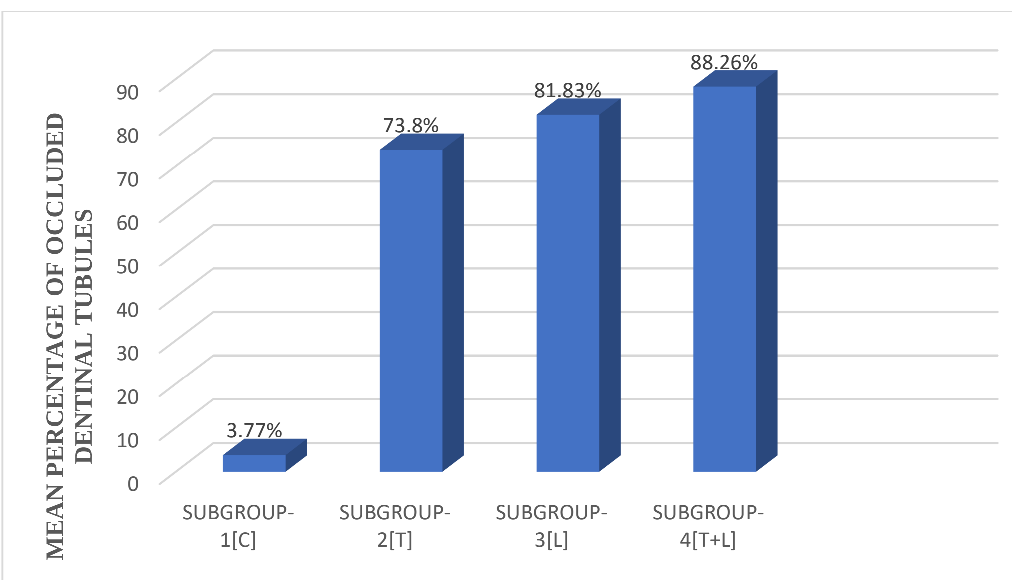


[POST CITRIC ACID CHALLENGE]

GRAPH-3- Comparative analysis of percentage of occluded dentinal tubular occlusion in all three test subgroups post citric acid challenge.



GRAPH-4 Mean percentage of occluded dentinal tubules in control and test subgroups post citric acid challenge.



DISCUSSION

Dentinal hypersensitivity (DH) is a typical clinical circumstance. Any extreme pain that a patient experiences in response to a particular stimulus is referred to as DH, regardless of the location (buccal, lingual, palatal, or occlusal).[1] True hypersensitivity can arise from pulpal inflammation, which displays the clinical signs of permanent pulpitis, such as intense and persistent pain, as opposed to the normal short, acute pain of Dentinal hypersensitivity.[1]

Based on the studies, DH is developed in two phases:

1. Lesion localization

2. Lesion initiation

In the first phase, dentinal tubules, due to loss of enamels, are exposed by attrition, abrasion, erosion, and abfraction. However, dentinal exposure mostly occurs due to gingival recession along with the loss of cementum on the root surface of canines and premolars on the buccal surface. It is worth noticing that not all the exposed dentins are sensitive. However, their calcified smear layer, as compared to non-sensitive dentin, is thin and this leads to an increase in the fluid movement and consequently the pain response.[32]

In the second phase, for the exposed dentin to be sensitized, the tubular plugs and the smear layer are removed and consequently, dentinal tubules and pulp are exposed to the external environment. Plug and smear layer on the surface of exposed dentin are composed of elements of protein and sediments which are derived from salivary calcium phosphates and seal the dentinal tubules inconsistently and transiently.[32] The findings of laboratory research indicate that both mechanical and chemical factors are effective in removing the smear layer from the dentinal tubules and making it patent.[32]

Since the dentin, which is sensitive to certain stimuli, is permeable through its thickness, any treatment that lessens dentinal permeability must also lessen DH. However, occasionally, even after effective tubule occlusion, DH can remain unaffected, suggesting that other mechanisms other than or in addition to the hydrodynamic mechanism are involved in nerve activation. Hence, dentists and patients have long struggled with dentin hypersensitivity.[33]

Saliva is said to play an important role in the remineralisation process of enamel. NovaMin® (calcium sodium phosphosilicate) (CSPS) is a material that acts on the same principle. NovaMin® is made from the same bioactive material, which is used in the bone regeneration process and same minerals which are naturally found in saliva.[14]

NovaMin® (calcium sodium phosphosilicate) as it is referred to under its trade name, is an inorganic, amorphous melt-derived glass compound that contains only calcium, sodium, phosphate, and silica and is one such product which when exposed to water/body fluids (saliva), reacts instantly by releasing billions of mineral ions that become available to the natural remineralization process in the oral cavity.[14]

The results of the same have been confirmed by scanning electron microscope (SEM) analysis which show that application of NovaMin® (calcium sodium phosphosilicate) results in the formation of a calcium hydroxyapatite layer, which occludes the dentinal tubules. It deposits hydroxycarbonate apatite and reduces the possibility of reopening the dentinal tubules.[14]

Conventional treatment methods as described before have a disadvantage of having to be replaced regularly and repeatedly in order to achieve continuous pain relief due to consumption of acidic foods and aggressive tooth brushing habits that cause gradual removal of precipitations and superficial coatings of the treatment agents which are applied on to the tooth surface. The use of lasers might open up a new dimension and possibilities in the treatment of dentinal hypersensitivity.[34]

According to previous researches, the effect of laser on the treatment of DH is different and varies between 5-100% based on the type of laser and therapeutic parameters such as the laser beam, the amount of time spent on the use of laser; and the intensity of laser [35,36]. Different mechanisms of action have been proposed based on its effect on the dentin.

1. Occlusion through coagulation of the proteins of the fluid inside the dentinal tubules
2. Occlusion of tubules through partial sub-melting
3. Discharging of internal tubular nerve

This form of therapy is found to be more effective due to its proper usage and minimal negative impact. So far, there has been no report of adverse reactions or pulpal damage in the studies conducted. Thus, the use of laser in treatment of DH is both logical and acceptable.[35,36]

The use of lasers along with desensitizing agents such as fluorine, potassium salts, oxalates etc have shown a synergistic effect in the reduction of DH, hence, referring to this course of action, it was proven how the low-power lasers, including the GaAlAs diode laser with a wavelength between 780 and 900 nm, act on the nerve tissue, thus eliminating hypersensitivity.[3] The medium-power lasers, including Nd: YAG, CO_2 and Er: YAG laser, have proven to desensitize via narrowing of dentinal tubules.

Although it has been demonstrated that some dentifrices are able to reduce dentin permeability in vitro, there is little information regarding their effects under a simulated oral environment.[28]

Exposure to acid and saliva could reverse the permeability effect caused by the desensitizers and dentin bonding agents, an ideal desensitizing agent needs not only to reduce the dentin permeability, but also maintain the occlusion effects in the face of acid challenges and artificial saliva immersion as mentioned in the study by Addy et al.[37]

Despite the plethora of techniques and therapeutic alternatives discussed in the literature, no single or combination of treatments has yet been proven to be the gold standard.

The study was carried out with invitro study design with 30 dentinal discs in each group as follows Group 1Control [C], Group 2 Toothpaste[T], Group 3 Laser[L] and Group 4 Toothpaste and Laser [T+L] [FIGURE-8]. The parameter for the evaluation of dentinal tubule occlusion was measured via percentage of occlusion in 120 dentinal discs post treatment with desensitizing agent [TABLE 1] [GRAPH 1].

Mean, standard deviation and standard error of the percentage of occluded tubules in the all three test groups highly significant (p<0.01) [TABLE-2] [GRAPH 2]. Intergroups and intragroup comparisons were done using Tukey's post hoc test for the same and statistically evaluated for significance (p<0.01) [TABLE 3] [TABLE 4]. Further citric acid challenge for the same group of dentinal discs and was sub grouped as Subgroup 1[C], Subgroup 2[T], Subgroup 3[L] and Subgroup 4[T+L] and percentage of dentinal tubular occlusion was calculated [TABLE 5] [GRAPH 3]. Mean, standard deviation and standard error of the percentage of occluded tubules in the all three test groups significant (p<0.01) post citric acid challenge [TABLE 6] [GRAPH 4]. Inter subgroups and intra subgroup comparisons were done for the same subgroups using Tukey's post hoc test and statistically evaluated for significance at (p<0.01) [TABLE 7] [TABLE 8].

Mean percentage and standard deviation of dentinal tubular occlusion in Group-1[C] that is distilled water was found to be 3.77±2.81 indicating that maximum number of patent dentinal tubules were present in this group as the smear layer was removed by treating it with 0.5 M EDTA solution [FIGURE-11]. Patent dentinal tubules indicate no occlusion and hence can be taken as reference for comparison with the other three treatment group [FIGURE1]. Mean percentage and standard deviation of occluded dentinal tubules in treatment Group-2[T] was 91.13±5.83, for Group-3[L] was 93.1±5.20 and for Group-4[T+L] was 97.7±2.92 [TABLE 1] [GRAPH 1].

A statistically significant increase in mean percentage of dentinal tubule occlusion was seen in Group 1[C] 3.70 ± 2.81 and Group 2 [T] 91.13±5.83 (p-value<0.01) [TABLE 4]. The possible mechanism that has been confirmed about NovaMin® containing toothpaste by Andersson OH et al is that calcium sodium phosphosilicate forms a strong attraction to collagen.[11]As dentin consists of a high proportion of collagen, its proposed that NovaMin® will bind to exposed dentin surfaces, as well as physically occlude the dentin tubules. The interaction of NovaMin® with collagen has been studied by several researchers using in vitro models. These studies have shown that the initial reactivity of the NovaMin® particles is associated with the development of a surface negative charge, and that this enables binding to side groups on Type I collagen fibers.[39] When NovaMin® particles come into contact with an aqueous environment such as water or saliva, an immediate release of sodium ions occurs which increases the local environmental pH. This facilitates the rapid precipitation of a calcium phosphate hydroxycarbonate apatite layer following subsequent release of calcium and phosphate ions [FIGURE 12].[10] This mechanism was confirmed by Lorenzo et al where semiquantitative EDX compositional analyses using NovaMin® containing toothpaste showed an increase in Si/Ca/P (5.7/1.4/1) ratio from pre-treatment levels to (6.3/1.9/1) post treatment, suggesting an effective deposition of this solid phase on dentin surface.[38] In our study 91.13% of mean dentinal tubules occlusion can be attributed to the fact as per the research done by Shah et al in which NovaMin® dentifrice showed 95.58% dentinal tubules occlusion. This was also in accordance with Earl, et al. and Parkinson et al who worked on the physical and chemical characterization of dentin surface that were affected by treatment with pure NovaMin® and saliva substitutes. Using modern imaging and analytical techniques it was observed in in-vitro reactions of the NovaMin® that amorphous calcium sodium phosphosilicate was converted to crystalline hydroxyapatite thus occluding the dentinal tubules up to 95%. [13]

Damen and ten Cate et al gave another possible mechanism of action for NovaMin® that it plays a key role of soluble silica in the formation of the calcium phosphate mineral. The effect of soluble silica on the precipitation of calcium phosphates demonstrated that polymers of silicic acid increased the rate of precipitation of hydroxyapatite, even in the presence of inhibitors of hydroxyapatite.[39] A feature of NovaMin® particles is that they release silica into the local environment (at a concentration between 15 and 40 ppm); this is hypothesized to be one of the key enablers in the early stages of precipitation of calcium phosphate by providing a nucleating site.[39] Hench et al did computer modelling of the interactions of small silica chains with calcium and phosphate ions which showed that a three-member silica chain is optimal as a nucleating site for hydroxyapatite formation.[40]

Similarly, Skallevold et al in his study containing bioactive glass formulations (CSPS) provide therapeutic relief via occluding the dentinal tubules by binding to collagen fibers and depositing hydroxyapatite layer simultaneously. Hence, this mechanism is said to provide immediate relief after using NovaMin® containing dentifrice.[41]

Similar findings were observed in studies conducted by Wang and Burwell et al where NovaMin® was found to adhere to an exposed dentin surface and reacted with it to form a mineralized layer.[12]

Citric acid challenge findings

Dentinal tubules occlusion decreased from 91.13±5.83 in Group 2 [T] to 73.22±7.86 in Subgroup 2[T] [TABLE 6] [GRAPH 3] which can be attributed to the reason that NovaMin® containing dentifrice is more effective at a higher pH

[FIGURE-16]. Olley RC et al called it a surface level phenomenon in which as soon as pH decreases there is reduction in occlusion of dentinal tubules as there is demineralization of the (CSPS) components that leads to the opening of the dentinal tubules [42][FIGURE 16]. Similar finding has been confirmed by Zhejun W et al. in his study in which there was reduction in dentinal tubules occlusion post citric acid challenge.[29] However, Sneha A et al and Shaikh K et al. confirmed although its efficacy decreases in acidic pH but still it is more efficacious than arginine-containing dentifrice and BioMin® containing dentifrice. Therefore, repeated application of NovaMin® containing toothpaste can help achieve optimum results.[29]

A statistically significant increase in mean percentage of dentinal tubule occlusion was seen in Group 1[C] 3.70 ± 2.81 and Group 3[L] 93.13±5.20 (p-value<0.01) [TABLE 4][GRAPH 1]. The possible mechanism attributed for the same is that hydroxyapatite structure to melt on laser application, which resolidifies on cooling and forms larger size hydroxyapatite crystals as compared to initial structure, thus leading to partial or complete obliteration of dentinal tubules [FIGURE13].[17] In the present study, the irradiation parameters of GaAlAs laser are used with power of 1W and was found to be quite effective in occluding the dentinal tubules, with minimal hazardous effects like craters and cracks, whereas Study done by Lin et al has reported that power setting of 0.5W is ineffective and more than 1W can cause irreversible damage to pulp and dentin. 31 However, only the positive effect of GaAlAs laser on obliteration of dentinal tubules has been seen till now.[26]Matsumoto K. et al gave a treatment range of effectiveness from 53.3%–94.2% for the GaAlAs laser.[17]

On intragroup comparison between Group 2[T] and Group 3[L] mean percentage of dentinal tubules occlusion was found to be 91.13±5.83 and 93.13±5.20 respectively which was found to be statistically insignificant (p=0.31) stating that NovaMin® toothpaste and diode laser both had equal potency for occlusion of dentinal tubules [TABLE 4][GRAPH 2].

Attributing to the fact stated by Liu et al that NovaMin® is capable of sodium ion diffusion rapidly when in contact with any aqueous media. It causes a calcium and phosphate release along with rise in pH.[21] They act as reservoirs for calcium and phosphate ions which may be released over several days. Finally, the calcium-phosphate complexes are capable of crystallizing into hydroxycarbonate apatite whereas with diode laser produces energy that is absorbed by dentin's mineral content which includes carbonates and phosphates that leads to melting of the crystalline arrangement of patent dentinal tubules.[21] hence, occluding the dentinal tubules. [26]

Similar results to our study Lund et al, assessed the effectiveness of sodium fluoride gel and diode laser (infrared) for dentin hypersensitivity, proposed that there was no difference among the desensitizing toothpaste and diode laser group groups. However, Yilmaz et al, found that GaAlAs laser was more effective and had superior effect than the desensitizing dentifrice treatment approach (NaF).[20]

Fabio et al. used (GaAlAs) diode laser 970nm wavelength laser with an optical fiber (200 mm diameter). The specimens were irradiated with a frequency of 10 Hz in contact mode for 30 seconds and results showed significant dentinal tubule occlusion. Rajeswari et al, specimens were treated with a 980 nm GaAlAs diode laser (0.5W, non-contact mode with a distance of 2-4 mm and using a fiber of 320µm diameter) for 60 seconds showed higher number of dentin tubules occlusion.[43]

Citric acid challenge findings

Reduction in dentinal tubule occlusion post citric acid application was seen from Group 3[L] 93.10±5.20 to Subgroup 3[L] 81.83±5.30 p-value(<0.01) was attributed to study done by J. Yu et al which states laser application, melts dentin with reformation of recrystallized granules, and after solidification an uneven mosaic-fern like appearance is observed, when being exposed to acid, a dentin surface with a smoother appearance is formed the reason for this is that the mineral content of the peritubular dentin is higher than in the intertubular dentin, thereby dissolving more dentin when exposed to acid [FIGURE-17].[44] Yesim et al in their study confirmed diode laser undergoes increased disocclusion of blocked dentin tubules once subjected to citric acid. [44]

Thus, it has been proposed that desensitizing process using combination therapy that includes laser in conjunction with tubular occluding agents like fluoride varnishes and other agents compatible with the applied laser's wavelength, is proven to show beneficial effects in the treatment of DH. Research suggests that, in order to achieve long-term desensitizing effects, laser's property to melt the dentinal tubules intrinsically and NovaMin®'s ability to mechanically occlude the tubules from the outer surface of dentin have a collusive effect.[39]

A statistically significant increase in mean percentage of dentinal tubule occlusion was observed between Group 1[C] 3.70 ± 2.81 and Group 4 [T+L] 97.17± 2.92 (p<0.01) [TABLE 4] [GRAPH 2]. This can be attributed to the fact that the diode laser provides biostimulatory action, melting of peritubular dentinal contents and the NovaMin® toothpaste acts via chemical occlusion releasing calcium, sodium and phosphate and silica ions and thus forming surface coating and effectively occluding the dentinal tubules respectively [FIGURE-14]. LMS Al-Saud et al in his SEM study has shown similar occluding effect of diode laser when used along with a dentin desensitizing agent.[23]

Intragroup comparison between Group 2[T] 91.13±5.83 and Group 4[T+L] 97.17± 2.92 and Group 3[L] 93.1±5.20 and Group 4[T+L] 97.17± 2.92 (p-Value<0.01) [TABLE 3] [TABLE 4] showed maximum mean percentage of dentin tubule occlusion which was highly significant. Attributing to the fact that combination of agents along with laser application helps in forming a strongly adherent physiological, chemical and mechanical bond which is difficult to erode. Study conducted by Reddy et al[26] is in accordance to our findings, where Diode laser (Group III) showed a higher efficacy in occluding dentinal tubules when compared with desensitizing toothpastes, i.e., NovaMin® (Group I) and Pro-Argin (Group II) that was statistically significant. It was also observed that the efficacy of diode laser when combined with a desensitizing toothpaste (Group IV – NovaMin®+diode laser and Group V – Pro-Argin+ diode laser) was more when compared to Group III (diode laser) alone and was statistically significant thereby proving that combination therapy plays an active role in increasing the number of dentinal tubule occlusion. Results also showed that Group IV (Novamin + Diode laser) showed the highest percentage of complete occlusion of dentinal tubules when compared to Group V (Pro-Argin + Diode laser).[26]

Citric acid challenge findings

Comparison between Group 4[T+L] 97.17±2.92 and Subgroup 4 [T+L] 88.26±4.84 showed a reduction in dentinal tubular occlusion (p<0.01) which can be attributed to the fact that the efficacy of NovaMin® decreases in acidic pH and forms a globular surface around dentin and laser coating forms smoother surface around peritubular dentin which dissolves and disoccludes the blocked dentin tubules. But this does not occur to a larger extent with combination of NovaMin® containing toothpaste and diode laser as the double coating is difficult to erode from dentin surface [FIGURE-18]. Hence, the maximum amount of occlusion of dentinal tubules was seen in combination of NovaMin® containing toothpaste even after subjecting it to citric acid challenge.

This finding was also confirmed by Hsu et al. who evaluated the combined occluding effects of fluoride-containing dentin desensitizer and laser irradiation on human dentinal tubules and concluded that occluding agent can neither be dissolved by vitamin C containing solution nor removed by brushing.[49] Study done by Kumar and Mehta et al showed that combined application of laser along with fluoride desensitising agent increases the effectiveness of the treatment with and without citric acid challenge. The results were in agreement with our findings.[45]

However, Geraldo Martins et al and Bal et al in their studies state that diode laser and the dentifrice used were effective in reducing dentinal permeability, and the combination of the two treatments did not show better results than either one used alone because the diode laser already melts the dentin tubules leading to occlusion of tubules hence, application of desensitizing paste did not further provide any additional benefit. However, both the contradictory studies have not mentioned valid reasoning for the above.[46]

Therefore, within the limitation of our study it can be concluded that the use of combination therapy comprising of NovaMin® containing toothpaste and diode laser 940nm proved in occluding the dentinal tubules effectively via chemical and mechanical bonding process even at lower pH levels after being subjected to citric acid challenge. However, to confirm the results of our study, more in vivo clinical trials need to be carried out with a larger sample size of patients so as to confirm the exact efficacy of the desensitizing agents in treatment of dentinal hypersensitivity. Also, further research should be targeted towards newer agents that have equal potency in the treatment of dentinal hypersensitivity.

SUMMARY AND CONCLUSION

The present study was in-vitro study that aimed to compare the efficacy of diode laser (940 nm), commercially available toothpaste containing desensitizing agent and combination of both on the occlusion of dentinal tubules using scanning electron microscope.

A total of 120 dentinal discs of diameter 5mm and thickness 2mm was prepared from 120 healthy extracted teeth. The study was conducted in the ‘Department of Periodontics’, KAHER’S KLE V.K. Institute of Dental Sciences, Belagavi.

A total of 4 groups were made out of which one was take as control and the other three test groups. Amongst which Group 1[C] was control treated with distilled water Group 2 [T] with NovaMin® toothpaste, Group 3 [L] diode laser (940 nm) (GaAlAs-940nm) and Group 4[T+L] combination of both NovaMin® toothpaste and diode laser (940 nm) (GaAlAs-940nm) and were kept in artificial saliva and then were dried and SEM analysis was done followed by citric acid challenge and were sub grouped as Subgroup 1[C]-distilled water, Subgroup[T]- NovaMin® toothpaste, Subgroup 3[L] diode laser (940 nm) (GaAlAs-940nm) and Subgroup 4[T+L] combination of both NovaMin® toothpaste and diode laser (940 nm) (GaAlAs-940nm) and SEM analysis was done.

After SEM analysis the parameter for which was analysed was mean percentage of dentinal tubular occlusion in all the groups at two-point interval with artificial saliva and citric acid challenge. Mean percentage and standard deviation of occluded dentinal tubules in treatment Group-2[T] was 91.13±5.83, for Group-3[L] was 93.10±5.20 and for Group-4[T+L] was 97.70±2.92. Compared to group 2[T] and group 3[L] the percentage of occluded dentinal tubules was higher in group 4[T+L] that is 97.2%. The three test groups are statistically significant as the p-value was found to be <0.001. indicating that combination of both NovaMin® toothpaste and diode laser (940 nm) (GaAlAs-940nm) is most efficacious when kept inartificial saliva. Similarly post citric acid the mean percentage and standard deviation of occluded dentinal tubules in treatment subgroup-2[T] was 73.22±7.86, for subgroup-3[L] was 81.83±5.30 and for subgroup-4[T+L] was 88.26±4.84. p-value was found to be <0.001 indicating its statistically significant. Hence, even after citric acid application the most effective form of treatment modality for DH was Subgroup 4[T+L] combination of both NovaMin® toothpaste and diode laser (940 nm) (GaAlAs-940nm) with maximum amount of tubular occlusion.

Hence, from our study it can be concluded that the most effective treatment for dentinal hypersensitivity is the combination of NovaMin® toothpaste and diode laser (940 nm) (GaAlAs-940nm) followed by diode laser (940 nm) (GaAlAs-940nm) and NovaMin® toothpaste. Long term in vivo studies with larger sample size and newer agents should be targeted.

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ANNEXURES:

1. ETHICAL CLEARANCE CERTIFICATE

Research and Ethics Committee
KLE V K INSTITUTE OF DENTAL SCIENCES
KLE University

KLE UNIVERSITY

Accredited 'A' Grade by NAAC — Placed in Category 'A' by MHRD (GoI)

Nehru Nagar, Belagavi - 590 010, Karnataka State

☎: 0831-2470362
FAX: 0831-2470640

Web: http://www.kledental-bgm.edu.in
E-mail:principal@kledental-bgm.edu.in

Sl. No. : 1486

CERTIFICATE

This is to Certify that the synopsis titled Comparative Assessment of Diode Laser, Commercially available toothpaste containing Desensitizing Agent and Combination of both on the Occlusion of Dentinal Tubules using Scanning Electron Microscope – An Invitro Study *Submitted by*

Dr. **REG NO. IK0220005** ____________ *P. G. Student / Staff, Guided by* ____________ *from Department of* Periodontics *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 5/5/21

Member Secretary
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Chairman
Research and Ethical Committee
KLE VK Institute of Dental Sciences
Belgaum

2. BIOSTATISTICS CLEARANCE CERTIFICATE

KLE V.K. Institute of Dental Sciences
(A Constituent unit of KLE Academy of Higher Education & Research
Deemed-to-be-University u/s 3 of the UGC Act, 1956)
Nehru Nagar, Belagavi-590 010 INDIA
Re-Accredited 'A' grade by NAAC (2nd Cycle) & Placed in Category 'A' by MHRD (GoI)
☎ : 0831-2470362 Web: http://www.kledental-bgm.edu.in
FAX: 0831-2470640 E-mail: principal@kledental-bgm.edu.in

Biostatistics Clearance Certificate

This is to certify that the Biostatistics aspect of the Dissertation / Research work of **REG NO. IK0220005** Graduate Student, under the guidance of , Reader, Department of Periodontics entitled **"Comparative assessment of diode laser, commercially available toothpaste containing desensitizing agent and combination of both on the occlusion of dentinal tubules using scanning electron microscope – an invitro study."** has been done under my guidance and considered satisfactory.

Dr. J. B. Prasad.

Place: Belagavi

Date: 13/12/22

Name & Signature of Biostatistician

DEPARTMENT OF EPIDEMIOLOGY & BIOSTATISTICS • KAHER'S • BELAGAVI

3. PLAGIARISM CHECK REPORT

Scientific Correspondence and Review Committee

KLE VK Institute of Dental Sciences

A Constituent Unit of KLE Academy of Higher Education and Research
(Deemed-to-be-University u/s 3 of the UGC Act, 1956)
Nehru Nagar, Belagavi - 590 010, Karnataka State

Accredited 'A' Grade by NAAC (2nd Cycle) **Placed in Category 'A' by MHRD (GoI)**

☎: 0831-2470362 **Web: http://www.kledental-bgm.edu.in**
FAX: 0831-2470640 **E-mail:principal@kledental-bgm.edu.in**

Date : 26/12/2022 **Serial No. :** 142

PLAGIARISM CHECK REPORT

Name of the Applicant : **REG NO. IK0220005**
UG / PG / Ph.D / Staff : Post graduate student
Batch & Year : 2020 – 2023
Department : Periodontics

The soft copy of Research Work / Manuscript by **REG NO. IK0220005** entitled "comparative assessment of diode laser, commercially available tooth paste containing desensitising agent and combination of both on the occlusion of dentinal tubules using SEM – An invitro study" under the guidance of ……… …..has been submitted for Anti-Plagiarism check to the Scientific Correspondence & Review Committee of KLE VK Institute of Dental Sciences using "Turn-it-in" software.

The scan has been carried out and the scanned output reveals a Similarity Index of ……2……%, which is **within** / **not within** the acceptable limits of 10% as per the UGC guidelines.

Member Secretary
Scientific Correspondence and Review Committee
KLEVK Institute of Dental Sciences
KAHER-Belagavi

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Scientific Correspondence and Review Committee
KLEVK Institute of Dental Sciences
KAHER - Belagavi