



## Original Article

# Effect of CO<sub>2</sub> Laser and Selected Nanoparticles on The Microhardness of Human Dental Enamel In vitro Study

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## ABSTRACT

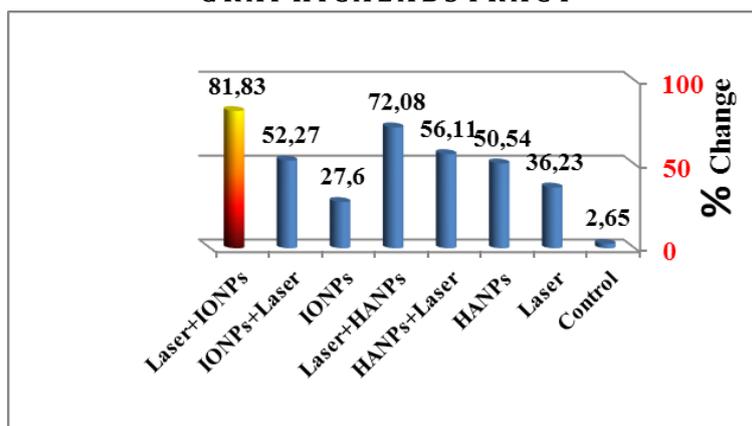
Lasers have been tested with positive findings for the suppression of incipient caries, and many theories have been offered for this phenomenon. Nevertheless, early caries lesion prevention and biomimetic treatment are still difficulties despite massive efforts to promote dental hygiene. Nanoparticles' exceptional qualities make them a promising biomaterial for a variety of medical and dental uses. There are a lot of exciting and promising uses for nanotechnology in the field of tissue repair and replacement, especially in dental mineralized tissues, thanks to the growing interest in this area. This research aimed to examine how certain nanoparticles and CO<sub>2</sub> laser radiation affected the microhardness of enamel. In order to conduct the microhardness test, 80 first premolars from the maxilla were randomly split into eight groups, one control group and seven study groups, each containing ten teeth. Each tooth's buccal (cheeky) side was standardized to have a circular window placed there, measuring 6 mm in diameter. The exposure period was 5 seconds in continuous wave (CW) mode, and the laser power was calculated to be 0.85 W using a unique equation. The concentration of the hydroxyapatite nanoparticles solution employed was 10%, whereas the concentration of the iron oxide nanoparticles solution was 12.5%. In this study, we evaluated the effects of certain agents on the microhardness of enamel before and after inducing a caries lesion by pH cycling techniques. Hardness variation was determined for each sample using a tailored equation. After demineralization, enamel microhardness values were found to be significantly lower across the board compared to healthy teeth' microhardness values. This was the case across all groups. After treatment with certain agents, there was a marked increase in enamel microhardness values for all groups, with a statistically highly significant difference ( $p < 0.001$ ). The groups treated with laser followed by hydroxyl apatite nanoparticles (HANPs) and those treated with laser followed by iron oxide nanoparticles (IONPs) saw the greatest increases in their microhardness values. Treatment of teeth samples with laser followed by iron oxide nanoparticles (IONPs) induced the greatest change in enamel microhardness, while treatment of teeth samples with IONPs resulted in the lowest change in enamel microhardness compared to all of the other agents. As a potential preventative intervention against dental cavities, enamel treatment with CO<sub>2</sub> laser, hydroxyapatite, and iron oxide nanoparticles might be examined.

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## GRAPHICAL ABSTRACT



## Introduction

People have been interested in the properties of light and how it might be employed in healing ever since the beginning of time. This fascination has persisted even today. The roots of this fascination go back to ancient times. The developments in physics that took place around the start of the twentieth century laid the framework for Albert Einstein's laser hypothesis, which eventually led to the development of this one-of-a-kind form of light in 1960. Almost immediately after that, researchers started investigating the possible use of laser technology in medical and dental care. The term "light amplification by stimulated emission of radiation" is an acronym for the word "laser," which is typically transcribed as "laser" [1]. The carbon dioxide laser, often known as the CO<sub>2</sub> laser, was one of the first gas lasers to be constructed. It is still considered to be one of the most valuable lasers in use today due to the wide variety of power levels that can be achieved with it as well as its relatively low cost [2]. Irradiation with a laser creates an interaction between light and the biological components of dental hard tissues, which results in heat formation; the effect of this thermal impact is modifications to the structure of the enamel, as well as changes to its chemical makeup [3]. *Enamel* is the name given to the coating of the tooth that is exposed to the surrounding environment. It is a calcified substance that is opaque despite its relatively thin thickness and hardness. The dentin located in the crown of the tooth is encased and safeguarded by this structure. Enamel

composition consists of 96 percent inorganic material, 4 percent organic material, and water. Enamel is the most mineralized tissue in the human body [4, 5]. Caries in the teeth is an infectious disease caused by bacteria that can be made worse by eating a diet that is heavy in carbohydrates. The primary process behind dental caries is demineralization, which can occur due to an attack by acids produced by bacteria such as *Streptococcus mutans* in dental plaque biofilms. Demineralization can occur as a result of an attack. The Gram-positive and a facultatively anaerobic bacterium known as *Streptococcus mutans* is a widespread inhabitant of the human oral cavity, where it plays a significant role in the progression of tooth decay [6, 7]. Atoms are clumped together to form nanoparticle, ranging in size from one nanometer to one hundred nanometers. It creates extremely helpful structures from individual atoms or molecules, which provides a new alternative and a possibly superior strategy in the prevention and treatment of dental caries, specifically in the remineralization of initial dental caries [8], which provides a new alternative and a possibly superior strategy in the prevention and treatment of dental caries, specifically in the remineralization of initial dental caries [8], which provides a new alternative and a possibly superior strategy in the prevention and treatment of dental caries, specifically [9]. The game-changing substance that is nanoparticles of hydroxyapatite is currently finding significant applications in the field of dentistry. They have the ability to restore demineralized areas of tooth

enamel by acting as a filler and repairing small holes and depressions on the enamel surface. They have a remarkable remineralizing effect on initial lesions of enamel and the ability to replace lost minerals to restore demineralized areas of enamel. In addition, they are capable of early remineralizing lesions that have occurred in the enamel since they have remineralizing actions on these lesions [10]. One of the many names that magnetic nanoparticles are given is iron oxide  $\text{Fe}_3\text{O}_4$  (magnetite), and it is one of the nanomaterials used in medicine. It is also one of the safest nanomaterials. In addition to their extraordinary magnetic characteristics, these nanoparticles also have an exceptional compatibility with biological systems [11]. Testing the influence of  $\text{CO}_2$  laser, hydroxyapatite, and iron oxide nanoparticles on the microhardness of human dental enamel may open the doors for employing them frequently in dental practice to prevent dental caries. If this is the case, it is possible that this testing will open the doors.

## **Materials and Methods**

Eighty maxillary first premolars were taken from Iraqi patients between the ages of 12 and 20 undergoing orthodontic treatment and then sorted into eight groups: one control group and seven study groups that were treated with selected agents, each group consisting of 10 teeth. This study was carried out after establishing the professional and ethical agreement by the concerned authority.

Control group: teeth with neither laser nor nanoparticles treatment.

Study group 1: teeth treated with 0.85W (CW)  $\text{CO}_2$  laser only.

Study group 2: teeth treated with 10% HANPs suspension solution.

Study group 3: teeth treated with (0.85W)  $\text{CO}_2$  laser followed by 10% HANPs suspension solution.

Study group 4: teeth treated with 10% HANPs suspension solution followed by (0.85W)  $\text{CO}_2$  laser.

Study group 5: teeth treated with 12.5% IONPs suspension solution.

Study group 6: teeth treated with (0.85W)  $\text{CO}_2$  laser followed by 12.5% IONPs suspension solution.

Study group 7: teeth treated with 12.5% IONPs suspension solution followed by (0.85W)  $\text{CO}_2$  laser.

On the buccal surface of each tooth, the positioning of a circular window measuring 6 millimeters in diameter was standardized [12]. Laser power was estimated to be 0.85 W, according to a special equation (Mathematical laboratory program), the exposure time was 5 seconds in a CW mode perpendicular to the enamel surface, with a spot size of 6mm, and the power density was ( $3\text{W}/\text{cm}^2$ ) in order to achieve chemical and morphological changes in the enamel surface without the risk of thermal damage (enamel surface temperature should not exceed  $42\text{ }^\circ\text{C}$ ) [13]. To make a 10% Hydroxyapatite nanoparticles suspension solution, in order to dissolve 100 grams of hydroxyapatite nanopowder, two molar of hydrochloric acid were utilized, and then 1 liter of deionized water was added. In order to achieve a pH of 7, sodium hydroxide, or NaOH, was used. As a result, a suspension solution that contains salts was produced. Salts were extracted from the solution using a separating funnel, and a suspension solution containing 10% HANPs was obtained [14, 15].

In contrast, the concentration of the iron oxide nanoparticles suspension solution was 12.5%. To make one liter of IONPs suspension solution with a concentration of 12.5%, dissolve 125 grams of IONPs powder in one liter of distilled water and then ultrasonify the mixture using a device capable of producing ultrasonic waves for twenty minutes to achieve a uniform suspension [16]. The pH cycling procedure was carried out by preparing demineralizing and remineralizing solutions. The demineralizing solution was comprised of (0.075 M/L acetic acid, 1.0 mM/L calcium chloride, and 2.0 mM/L potassium phosphate) at  $37\text{ }^\circ\text{C}$ , and the pH was adjusted to 4.3 by a pH meter. In contrast, the remineralizing solution was comprised of (150 mM/L potassium chloride [17]. Enamel microhardness was determined initially for normal enamel, after

induction of caries lesion by pH cycling process, and after treatment with selected agents by using digital micro-Vickers hardness tester, and the operations were carried out by the following steps [16, 17, 28, 29]:

The microhardness of normal enamel was first evaluated using a measuring device.

The demineralizing solution was poured into an incubator set to 37 degrees Celsius, and each tooth was submerged in it individually for six hours.

After being removed, each tooth was subjected to a two-minute rinsing with deionized water while running.

The individual tooth samples were then placed back into the incubator at 37 degrees Celsius for another 17 hours while treated with 20 milliliters of remineralizing solution.

After each tooth had been removed, a two-minute rinsing with deionized water was performed under running water for it before the process started again.

The four steps completed just before this one, were carried out once a day for ten days.

The specimens were then rinsed with deionized water, and a light microscope was used to detect any microscopic changes related to the formation of caries. Caries can be seen with the naked eye as a chalky white spot on the tooth after the tooth has been dried with airflow.

Following the production of a carious lesion with a pH cycling technique, the microhardness of the enamel was assessed.

The teeth were treated in accordance with the design of the group; either they were irradiated with a CO<sub>2</sub> laser using particular lasing parameters, or they were treated with a selected nanoparticle suspension solution. Both of these treatments were performed in a controlled environment (for four minutes by immersing each tooth separately in 20 ml of the selected agent solution; after that, each tooth was rinsed with deionized water for two minutes). The procedure of restoring the teeth in deionized water in the incubator at a temperature of 37 degrees Celsius for one night was carried out the following day, and it was carried out each day for

one week. The teeth were kept in the incubator at 37 degrees Celsius.

After applying the various chemicals, the samples were subjected to a subsequent examination in which the microhardness of the enamel was evaluated.

The microhardness of each specimen had its change in hardness, denoted by the letter "D," or the difference between its initial value, determined after a pH cycling procedure, and its final value, determined after treatment with certain agents. The mean of this difference, denoted by "D," was determined for each experimental group.

The ratio of the difference (D<sup>-</sup>) to the (Hvi<sup>-</sup>) was also calculated for each experimental group which represents the rate of change in hardness of the specimens [18].

$$D = H_{vf} - H_{vi} \quad (1)$$

$$(D^-) = \sum (D) / n \quad (2)$$

$$(Hvi^-) = \sum (Hvi) / n \quad (3)$$

$$\% \text{ change in the hardness values (CH)} = (D^-) / (Hvi^-) \times 100 \quad (4)$$

## Results and Discussion

After demineralization and subsequent treatment with various chemicals, the mean microhardness values of the sound enamel surfaces are presented in the following [Table 1](#). After demineralization, enamel microhardness values were significantly lower across all groups compared to healthy teeth' microhardness values. This was the case regardless of whether the teeth were extracted or not. Following treatment with certain agents, there was a discernible rise in the microhardness values of the groups administered laser, HANPs, HANPs + laser, IONPs, and IONPs + laser, respectively. While there was a significant rise in microhardness values for the group treated with laser and HANPs and the group treated with laser and IONPs, the former showed a greater increase. The results of an ANOVA test revealed that, during both the sound and demineralization stages, there were no statistically significant differences in the microhardness values found among the four groups (p less than 0.05). In contrast, statistically very significant variations were found during the

remineralization stage. According to an ANOVA test's findings, there were no statistically significant variations in microhardness values were discovered between the various groups ( $p < 0.05$ ). This was true for both the sound and demineralization stages. Statistically, highly significant variations between the groups were found when looking at the remineralization stage ( $p < 0.001$ ). The microhardness of enamel is depicted in Table 2, which uses repeated measurements to show the progression through the three stages. According to the ANOVA test results, there was a statistically highly significant difference was found between the three stages contained within the same group for each group ( $p < 0.001$ ). The results of additional statistical analysis are presented in Table 3, where they were utilized to demonstrate the mean difference between the stages that belong to the same group. Although there was a statistically insignificant difference between the demineralization and remineralization stages for the control group ( $p < 0.05$ ), there was a highly significant difference between all three stages for

all other groups ( $p < 0.001$ ). Multiple comparisons of microhardness values for all groups in the remineralization stage can be seen in Table 4. It was discovered that there was a statistically highly significant difference in the microhardness values of the control group when compared with the group treated with (laser + HANPs and laser + IONPs) ( $p < 0.001$ ), and this difference was found between the two groups. In contrast, there was a statistically significant difference between the groups treated with laser and those treated with laser plus HANPs or laser plus IONPs ( $p < 0.05$ ) in the group that was treated with laser. It was shown that there was a statistically significant difference between the groups treated with laser + HANPs, IONPs, and laser + IONPs ( $p < 0.05$ ) when comparing the groups treated with laser + HANPs, IONPs, and laser + HANPs. When compared with the group treated with IONPs and laser simultaneously, the groups treated with IONPs alone exhibited no statistically significant difference ( $p > 0.05$ ). It was found that there were no significant differences between the other groups ( $p > 0.05$ ).

**Table 1:** Microhardness values of enamel surfaces treated with different agents with statistical test

Groups	Sound stage	Demineralization stage	Remineralization stage
	(Mean $\pm$ SD)	(Mean $\pm$ SD)	(Mean $\pm$ SD)
Control	390.06 $\pm$ 70.51	103.41 $\pm$ 22.51	106.15 $\pm$ 22.72
Laser	367.96 $\pm$ 43.83	82.91 $\pm$ 15.81	112.95 $\pm$ 14.04
HANPs	341.15 $\pm$ 51.31	80.61 $\pm$ 18.75	121.35 $\pm$ 15.12
HANPs +Laser	357.83 $\pm$ 61.35	79.35 $\pm$ 19.50	123.87 $\pm$ 18.01
Laser +HANPs	347.42 $\pm$ 72.09	84.42 $\pm$ 22.48	145.27 $\pm$ 26.18
IONPs	373.56 $\pm$ 52.65	88.58 $\pm$ 24.24	113.03 $\pm$ 23.85
IONPs +Laser	332.07 $\pm$ 42.76	76.28 $\pm$ 18.33	116.15 $\pm$ 19.67
Laser+ IONPs	329.67 $\pm$ 35.43	80.29 $\pm$ 15.03	145.99 $\pm$ 19.22
Statistical test	F= 1.487 df= 7 P= 0.186	F= 1.831 df= 7 P= 0.094	F= 5.439 df= 7 P= 0.000 <sup>a</sup>

<sup>a</sup>Highly Significant, SD = Standard Deviation, df = degree of freedom

**Table 2:** Repeated measure ANOVA of enamel microhardness values for the three stages for each group

Group	Statistical test	
	F	p-value
Control	232.829	0.000 <sup>a</sup>
Laser	337.694	0.000 <sup>a</sup>
HANPs	386.533	0.000 <sup>a</sup>
HANPs+Laser	451.436	0.000 <sup>a</sup>
Laser+HANPs	623.267	0.000 <sup>a</sup>
IONPs	302.048	0.000 <sup>a</sup>
IONPs+Laser	371.402	0.000 <sup>a</sup>
Laser+IONPs	672.444	0.000 <sup>a</sup>

<sup>a</sup>Highly significant

**Table 3:** Multiple comparisons of enamel microhardness values for the three stages for each group

Group	Multiple comparisons		MD	P-value
Control	Sound	Remineralization	283.91	0.000 <sup>a</sup>
	Demineralization	Remineralization	-2.74	0.197
	Sound	Remineralization	255.01	0.000 <sup>a</sup>
Laser	Demineralization	Remineralization	-30.04	0.000 <sup>a</sup>
	Sound	Remineralization	219.80	0.000 <sup>a</sup>
	Demineralization	Remineralization	-40.74	0.000 <sup>a</sup>
HANPs+Laser	Sound	Remineralization	233.96	0.000 <sup>a</sup>
	Demineralization	Remineralization	-44.52	0.000 <sup>a</sup>
Laser+HANPs	Sound	Remineralization	202.15	0.000 <sup>a</sup>
	Demineralization	Remineralization	-60.85	0.000 <sup>a</sup>
IONPs	Sound	Remineralization	260.53	0.000 <sup>a</sup>
	Demineralization	Remineralization	-24.45	0.000 <sup>a</sup>
IONPs+Laser	Sound	Remineralization	215.92	0.000 <sup>a</sup>
	Demineralization	Remineralization	-39.87	0.000 <sup>a</sup>
Laser+IONPs	Sound	Remineralization	183.68	0.000 <sup>a</sup>
	Demineralization	Remineralization	-65.70	0.000 <sup>a</sup>

<sup>a</sup>Highly significant MD= Mean Difference

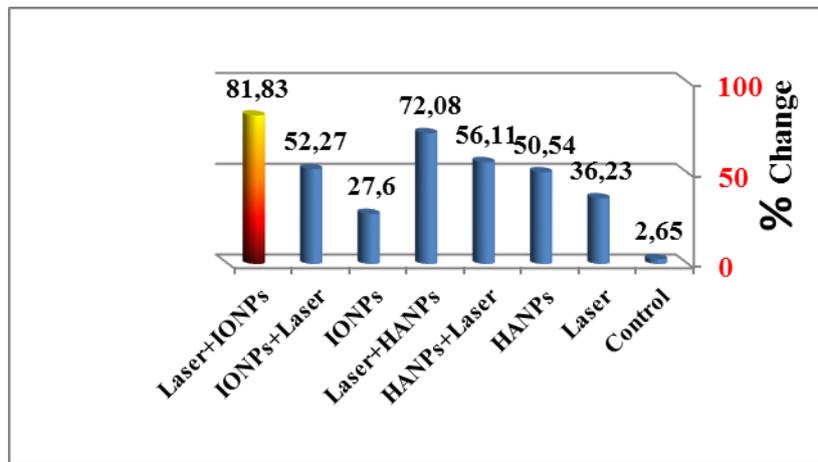
Figure 1 demonstrates the changes in microhardness values following treatment with various chemicals calculated using a specific equation. Compared to other agents, the values from this figure show a relatively small change in the microhardness for the control (sample teeth treated with deionized water). Compared to all other agents, treatment of teeth samples with IONPs resulted in the lowest change in the microhardness, while treatment of teeth samples with laser + IONPs generated the biggest change. In this study, the microhardness of enamel served as a surrogate for the degree of demineralization caused by the lesion [19]. This result was achieved by employing Vicker's method, which had been demonstrated successful in earlier studies. After pH cycling, all groups had a statistically significant decrease in the microhardness of the enamel surface. This showed that enamel demineralization and the beginning of a carious lesion had occurred.

Because demineralization happens when the pH of the surrounding environment falls below the critical PH (5.5), an acidic medium is created; this acidic medium causes the minerals of the tooth, primarily calcium and phosphorous, to move outward, leaving behind micro pores and reducing the tooth's hardness [20]. When the microhardness of the teeth was measured before and after laser treatment, the comparison revealed that the teeth in the laser-only group had a statistically significant increase in their microhardness. This may be explained by the fact that the absorption peak of hydroxyapatite crystal, the primary component of dental enamel, is in resonance with the wavelength of the CO<sub>2</sub> laser, which is then strongly absorbed and efficiently converted to heat without harming the underlying or surrounding tissues. In other words, the absorption peak of hydroxyapatite crystal is in resonance with the wavelength of the laser [21].

**Table 4:** Multiple comparisons of microhardness values for all groups in remineralization stage by using Tukey HSD test

Groups		Laser	HANPs	HANPs+ Laser	Laser+ HANPs	IONPs	IONPs+ Laser	Laser + IONPs
Control	MD	-6.80	-15.20	-17.72	-39.12	-6.88	-10.00	-39.84
	P-value	0.995	0.700	0.517	0.001 <sup>b</sup>	0.995	0.954	0.001 <sup>b</sup>
Laser	MD	-	-8.40	-10.92	-32.32	-0.08	-3.20	-33.04
	P-value	-	0.982	0.927	0.014 <sup>a</sup>	1.000	1.000	0.011 <sup>a</sup>
HANPs	MD	-	-	-2.52	-23.92	8.32	5.20	-24.64
	P-value	-	-	1.000	0.158	0.983	0.999	0.133
HANPs+Laser	MD	-	-	-	-21.40	10.84	7.72	-22.12
	P-value	-	-	-	0.275	0.930	0.989	0.237
Laser+HANPs	MD	-	-	-	-	32.24	29.12	-7.2
	P-value	-	-	-	-	0.014 <sup>a</sup>	0.039 <sup>a</sup>	1.000
IONPs	MD	-	-	-	-	-	-3.12	-32.96
	P-value	-	-	-	-	-	1.000	0.011 <sup>a</sup>
IONPs+Laser	MD	-	-	-	-	-	-	-29.84
	P-value	-	-	-	-	-	-	0.031 <sup>a</sup>

<sup>a</sup> Significant, <sup>b</sup>Highly significant, MD= Mean Difference



**Figure 1:** Changes in the microhardness values (%) after treatment with selected agents

The difference between the two groups is statistically significant when comparing the microhardness values before and after HANP treatment. This is because HANPs rehardens the softened enamel through the gradual deposition and re-precipitation of the mineral from the hyper-saturated solution of these ions on the enamel surface, which nucleates in the demineralized area to form an intact superficial layer on the enamel surface [22]. The additive effect of laser irradiation's melting and re-solidification on the group treated with HANPs afterward may be responsible for the statistically significant increase in microhardness measured after the laser was applied to the sample. Furthermore, laser irradiation of the enamel

surface promotes ion joining of the mineralizing agent [23, 24]. There was a statistically significant increase in microhardness values between the demineralization stage and the stage where laser and HANPs were used. One probable explanation is that the micro gaps (holes) created by the laser irradiation would be filled with the HANPs after they were applied, making it easier to incorporate the ions.

The microhardness mean values for the IONP-treated group rose significantly from the demineralization phase. This may be due to the fact that iron ions are incorporated into the structure of tooth enamel; however, the mechanism of their acquisition is still not fully understood; however, the incorporation of iron

ions results in the lower relative content of carbonate (in which carbonate is positively associated with dental caries and lower microhardness), which will have a beneficial effect on the physical and chemical properties of enamel [25-32]. compared to the demineralization phase, the microhardness mean values for the group treated with IONPs followed by laser were significantly higher. Laser irradiation causes enamel to melt and re-solidify, and the presence of iron ions in the outer enamel structure may have a synergistic impact. The results indicated the greatest average increase in microhardness relative to the demineralization stage for the group treated with laser and then IONPs. Laser irradiation, when applied first to the outer enamel structure, creates microvoids. These micro spaces provided a sanctuary for the assimilation of a large amount of IONPs, which may account for the observed effect.

Because of the consistency of the examination, the microhardness scores of teeth that were healthy teeth and those in diverse stages of demineralization were not substantially different from one another (the same type of teeth used, same tooth surface examined, and the same PH cycling method). During the entirety of the remineralization phase, statistically, significant differences were observed between the groups. These differences may be attributable to differences in the rehardening and remineralizing capabilities of the utilized agents and the order in which they were applied. There was a statistically significant difference between the microhardness values of the laser + HANPs and laser + IONPs groups and the control group, which is consistent with the findings of Ibrahim's study from 2013 [27], in which she found that irradiation of the enamel surface, followed by the application of a remineralizing agent (fluoride), will cause a better uptake by the enamel surface. In addition, there was a statistically significant difference between the microhardness Due to the synergistic action of both agents, which increased microhardness values more than when the laser was employed alone, a statistically significant difference was shown when comparing the laser-treated group with the laser-plus-HANPs group

and the laser-plus-IONPs group with the laser-treated group. This difference was demonstrated when comparing the laser-treated group with the laser-plus-HANPs group and the laser-plus-IONPs group with the laser-treated group. It is possible that the additive effect of the two agents, which elevated microhardness values more than either agent could have done on its own, contributed to the statistically significant difference that was observed between the groups treated with laser and HANPs and those treated with IONPs [33-40]. There were statistically significant differences between the groups when comparing the laser + HANPs group to the IONPs + laser group, as well as when comparing the laser + IONPs group to the IONPs group and the IONPs + laser group. This may be because researchers recently found that irradiating the enamel surface with a laser before applying a remineralizing chemical makes it more effective at absorbing the substance.

The microhardness of dental enamel was found to be raised by all agents tested, although the values still aren't anywhere near what they were in healthy teeth. Perhaps the application time utilized in this study is to blame. The average treatment time per tooth was four minutes, seven days per week. More research will be necessary to discover if the microhardness levels return to normal or are further increased if the treatment duration is extended from a week to several weeks. Microhardness values were measured before and after treatment with each agent using the same equations and found to change the most in the laser + IONPs group, then the laser + HANPs group, the HANPs + laser group, the HANPs group, the IONPs group, and finally the control group (de-ionized water), which changed the least. Since no previous research had employed this particular combination of drugs, the results of the current study cannot be compared to those of other investigations.

## **Conclusion**

A marked increase in enamel microhardness values for all groups with a statistically highly significant difference, with the maximum increase in microhardness values for the group treated with laser + hydroxyapatite nanoparticles

(HANPs) and the group treated with laser + iron oxide nanoparticles (IONPs). Treatment of teeth samples with laser + IONPs caused the biggest change in enamel microhardness, while treatment with IONPs resulted in the lowest among all other agents.

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### Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

### Conflict of Interest

The author declared that they have no conflict of interest.

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