



Effect of Er, Cr, Ysgg Laser on ‘Enamel Remineralisation’ Using Cpp – Acp, Nano – Hydroxyapatite & Zinc - Hydroxyapatite Containing Paste After ‘Enamel Erosion’ Caused by Carbonated Soft Drink in Permanent Teeth - A Comparative Invitro Study

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ABSTRACT:

Introduction : Dental erosion is becoming increasingly common due to modern lifestyles and dietary choices, particularly the high consumption of carbonated soft drinks. Traditional fluoride-based treatments may not be sufficient; therefore, there is growing interest in biomimetic agents such as (CPP-ACP), Nano-hydroxyapatite (n-HAP), and zinc-hydroxyapatite (Zn-HAP). This study investigates how combining Er,Cr:YSGG laser therapy with these agents enhances enamel resistance and repair, offering a less invasive method for treating early cases of enamel erosion.

Methods : Seventy human premolar teeth were used in the study. All the teeth were simultaneously treated with erosive drink and remineralising agent. They were then divided into seven groups: one control group (distilled water), and six experimental groups using CPP-ACP, n-HAP, Zn-HAP, and each of these combined with Er,Cr:YSGG laser treatment. Remineralisation effectiveness was assessed using Vickers microhardness testing and confocal scanning laser microscopy. Statistical analysis was performed using ANOVA with post hoc tests ($p < 0.05$).

Results : All groups showed reduced microhardness compared to the baseline ($p < 0.01$). The control group exhibited the greatest hardness loss (406 to 122.5). CPP-ACP reduced the loss to 229.3, n-HAP to 284.6, and Zn-HAP to 271.3. When combined with laser treatment, remineralisation improved significantly. The best results were observed with n-HAP plus laser, achieving the highest final hardness of 326.1.

Conclusions : The combination of Er,Cr:YSGG laser with biomimetic agents such as CPP-ACP, n-HAP, and Zn-HAP significantly enhances enamel remineralisation. This approach provides a conservative, fluoride-free option for managing early enamel erosion.

1. INTRODUCTION

Dental erosion, a non-infectious loss of tooth structure caused by chemical processes, has become a significant dental concern due to lifestyle and dietary changes. The condition is strongly

associated with the increasing consumption of acidic beverages such as soft drinks, sports drinks, and fruit juices. Studies have reported that the average annual consumption of carbonated beverages was approximately 68 gallons per person



in 2004. Consequently, dental erosion is becoming more prevalent in today's population.^[1,4]

Early-stage erosive lesions require conservative treatment strategies, including remineralising agents. High levels of fluoride may cause adverse effects, prompting the search for alternative treatments that function independently of fluoride.^[4,12]

- **Casein phosphopeptide (CPP):** A bioactive substance derived from milk that stabilises calcium phosphate clusters on the tooth surface. CPP-ACP (GC Tooth Mousse) is a widely studied fluoride-free formulation with proven remineralisation properties.^[8]
- **Nano-hydroxyapatite (n-HAP):** A biomimetic material that closely resembles natural enamel and dentin. It releases calcium and phosphate ions under acidic conditions, penetrates enamel micropores, and supports subsurface remineralisation. Unlike fluoride, which primarily acts on the enamel surface, n-HAP can repair deeper lesions.^[13,14]
- **Zinc-hydroxyapatite (Zn-HAP):** Another biomimetic material that mimics enamel composition. It forms a protective layer on the enamel surface, shielding it from acidic challenges. Products such as BioRepair, containing Zn-HAP, have demonstrated effective remineralisation and prevention of further demineralisation.^[3,5]

The **Er,Cr:YSGG laser**, operating at a wavelength of 2780 nm, is well absorbed by water and hydroxyl groups in hydroxyapatite. This enables the laser to create microspaces that trap ions, reduce acid impact, and decrease sensitivity. It can induce chemical and structural changes in enamel without excessive heat generation. Previous studies suggest that combining CPP-ACP with laser treatment enhances surface hardness. This study evaluates the

combined effects of Er,Cr:YSGG laser and biomimetic pastes on enamel erosion caused by carbonated drinks.^[7,8]

2. OBJECTIVES

To compare and evaluate the ability of Er,Cr:YSGG laser combined with CPP-ACP, nano-hydroxyapatite, and zinc-hydroxyapatite pastes to promote remineralisation and increase remineralisation depth in artificially induced enamel erosion caused by carbonated soft drinks, using surface microhardness analysis and confocal scanning laser microscopy.^[9]

3. METHODS

Seventy non-carious, healthy human permanent premolar teeth were collected from the Department of Oral and Maxillofacial Surgery at RajaRajeshwari Dental College and Hospital, following specific inclusion and exclusion criteria.

- **Inclusion:** Teeth with smooth, intact buccal enamel.
- **Exclusion:** Teeth with cracks, fractures, hypoplasia, fluorosis, cavities, carious lesions, other non-carious lesions, anomalies, or restorations.

Collection and Storage: A total of 70 healthy, intact permanent premolar teeth were extracted for orthodontic or periodontal reasons. The teeth were cleaned using an ultrasonic scaler and stored in 0.1% thymol solution to maintain sterility until further processing.

Enamel window preparation

All the teeth were sectioned to separate crown from the root portion using a diamond disc in an electric motor with straight headpiece. The obtained samples will be again sectioned in mesio-distal direction along the central groove and the buccal halves were used for the study. Then the surface of the teeth were polished with SiC paper and a diamond polishing paste to achieve uniform



characteristics. The samples were implanted in acrylic blocks such that the buccal surface are visible. A zone of 5mm x 5mm window was marked and glazed with nail varnish excluding the window.^[4]

Base line microhardness test

All the samples were subjected to Vickers microhardness testing machine (VMT) at the rate of 100g load for 10seconds. the average microhardness of specimen were determined from the three indentations to avoid any discrepancy.^[4]

The prepared samples were then divided into respective groups :

GROUP 1: Distilled water

GROUP 2: CPP – ACP

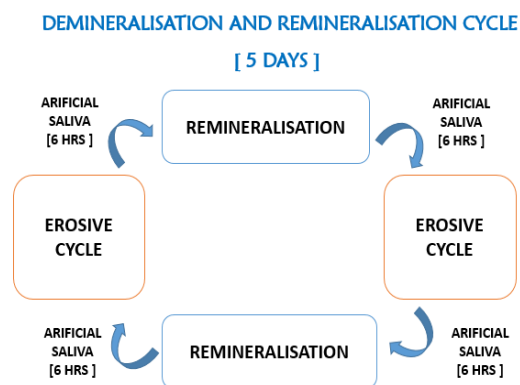
GROUP 3: Nano – hydroxyapatite

GROUP 4: Zinc – hydroxyapatite

GROUP 5: CPP – ACP + Er, Cr, YSGG

GROUP 6: Nano -HAP + Er, Cr, YSGG

GROUP 7: Zinc – HAP + Er, Cr, YSGG



Erosive cycle

The teeth were immersed in aerated drink [30 ml per sample, refrigerated, freshly opened, pH < 3] for 5 minutes, followed by immersing in artificial saliva for 6 hours before application of any remineralising agent or laser irradiation, and was done twice a day for 5 days.^[12]

Group 1 is treated with distilled water [control group](twice a day for 5 min in between the erosive cycle for 5 days), and placed back in artificial saliva.^[12]

Application of Remineralising agent

After 6 hrs of storage in artificial saliva, a pea sized remineralising agents of group 2, 3 & 4 were smeared evenly on all the surface with a cotton bud and left undisturbed for 3 minutes^[5], followed by rinsing with distilled water and again followed by storage in artificial saliva for 6 hours and the cycle was repeated twice a day for 5 days.^[12]

Laser irradiation

In case of group 5, 6, & 7 where laser is used, the teeth after removal from artificial saliva should be air dried for 3seconds, followed by application of respective remineralising agent and was immediately irradiated using Er, Cr YSGG laser.^[8]

Laser parameters

Power: 0.75 W

Energy density: 8.5 J/cm²

Energy per pulse : 12.5mJ

Pulse width : 140 μ s [H mode]

Frequency : 20 Hz [without air or water cooling]

Teeth after removal from artificial saliva, rinsed and air dried followed by application of remineralising agent and subjected to laser application. Laser tip to be perpendicular to the tooth surface with distance of 1mm from the teeth surface [rubber stopper to be used]. Each tooth to be radiated horizontally and vertically. The handpiece moved slowly for 10 seconds in each direction to promote homogenous radiation covering the whole surface. (regular inspection of laser tips, serrated tip was replaced and contaminated tip was cleaned by cotton moistened with alcohol).^[8] followed by rinsing with distilled



water and again followed by storage in artificial saliva for 6 hours and the cycle is repeated twice a day for 5 days.^[12]

Final microhardness test

All the samples will be subjected to Vickers microhardness testing machine (VMT) at the rate of 100g load for 10 seconds. The average microhardness of specimen will be determined from the three indentations to avoid any discrepancy.^[4]

Remineralising potential

The difference between the baseline and final microhardness values determines the remineralisation potential of the remineralising agents combined with or without laser irradiation^[8]

Remineralising depth

All the samples will be washed with distilled water, stained with 1% Rhodamine B solution and analysed using Confocal Scanning Laser Microscopy to determine the remineralising depth of the remineralising agents combined with or without laser irradiation^[9]

4. RESULTS

Based on the Vickers microhardness measurements, all intervention groups demonstrated statistically significant reductions in microhardness values from baseline ($p < 0.001$) for all pairs. The control group showed the most dramatic decrease from 406.030 ± 17.2006 to 122.560 ± 18.4219 , representing the greatest loss of surface hardness. Among the treatment groups, CPP-ACP demonstrated substantial remineralization efficacy, reducing hardness loss from 365.920 ± 26.8021 to 229.340 ± 12.2719 . The N-HAP groups showed comparable performance with final value 284.620 ± 11.1859 , while Zn-HAP achieved 271.330 ± 14.5793 . Laser-assisted treatments generally showed enhanced remineralization compared to their non-laser counterparts, with CPP-ACP + laser (283.720 ± 7.1737), and Zn-HAP + laser (304.480 ± 10.5744) demonstrating improved hardness retention, though N-HAP + laser showed the highest final hardness value of 326.150 ± 19.4938 among all treatment groups

Table.1- Comparison of Pre and Post Vickers Microhardness values of Intervention groups

Groups	BASELINE		FINAL		p-value [†]
	Mean	Std. Deviation	Mean	Std. Deviation	
CONTROL	406.030	17.2006	122.560	18.4219	<0.001 *
CPP-ACP	365.920	26.8021	229.340	12.2719	<0.001 *
N-HAP	358.020	26.2544	284.620	11.1859	<0.001 *
ZN-HAP	368.530	18.2224	271.330	14.5793	<0.001 *
CPP -ACP LASER	376.410	35.5529	283.720	7.1737	<0.001 *
N- HAP LASER	375.180	13.5784	326.150	19.4938	<0.001 *
Zn- HAP LASER	357.350	21.9154	304.480	10.5744	<0.001 *

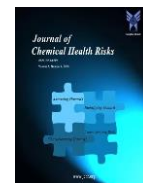


The ANOVA analysis revealed significant differences between intervention groups at both baseline and post-treatment measurements ($p=0.008$ and $p<0.001$, respectively). At baseline, despite statistical significance, the microhardness values were relatively comparable across all groups, ranging from 357.350 ± 21.9154 (laser Zn-HAP) to 406.030 ± 17.2006 (control), indicating successful randomization. However, post-treatment measurements demonstrated substantial inter-group differences, with the untreated control group showing the lowest hardness value of 122.560 ± 18.4219 , significantly different from all treatment groups. Among the interventions, CPP-ACP alone showed the least remineralization effect (229.340 ± 12.2719), while all other treatments demonstrated superior performance. The nano-hydroxyapatite combined with laser therapy achieved the highest post-treatment hardness value (326.150 ± 19.4938), followed by laser-assisted Zn-HAP (304.480 ± 10.5744), N-HAP alone (284.620 ± 11.1859), and laser-assisted CPP-ACP (283.720 ± 7.1737). Zinc-substituted hydroxyapatite without laser showed intermediate value of 271.330 ± 14.5793 , demonstrating that laser enhancement generally improved remineralization outcomes across different biomaterial platforms.

The post hoc Tukey analysis revealed significant pairwise differences between all intervention

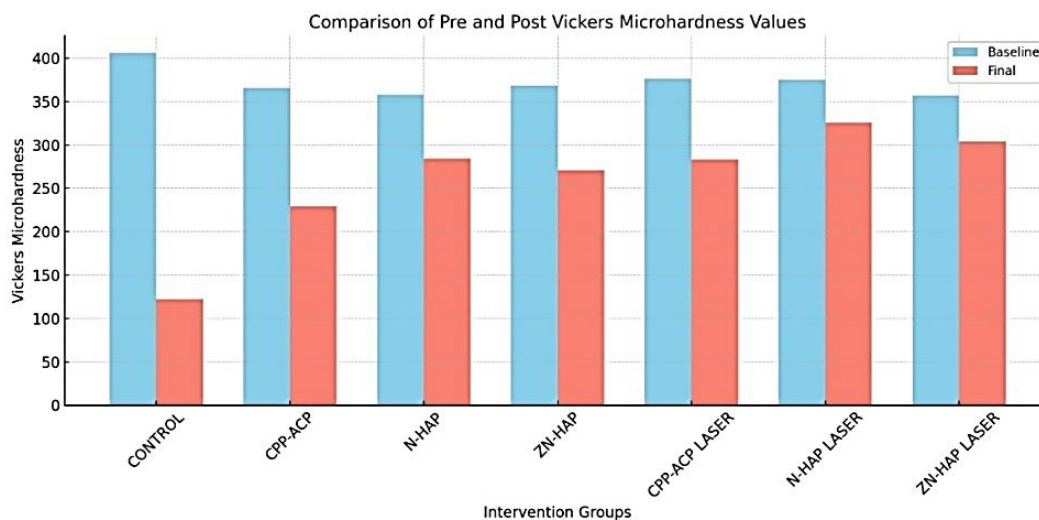
groups at both baseline and post-treatment phases ($p<0.05$). At baseline, the control group demonstrated significantly higher microhardness values compared to all treatment groups, with mean differences ranging from 29.62 (vs laser CPP-ACP) to 48.68 (vs laser Zn-HAP), indicating that despite randomization, the control specimens had inherently higher initial hardness values. Post-treatment comparisons showed dramatic differences, with the control group exhibiting significantly lower hardness than all intervention groups, with mean differences ranging from -106.78 (vs CPP-ACP) to -203.59 (vs laser N-HAP). Among the treatment modalities, laser-assisted nano-hydroxyapatite demonstrated superior performance, showing significantly higher hardness values compared to all other interventions, with mean differences of 96.81 (vs CPP-ACP), 41.53 (vs N-HAP alone), 54.82 (vs Zn-HAP), 42.43 (vs laser CPP-ACP), and 21.67 (vs laser Zn-HAP). The laser-enhanced treatments generally outperformed their non-laser counterparts, with CPP-ACP being the least effective intervention among all treatment groups, showing significantly lower hardness values compared to most other treatments, thereby establishing a clear hierarchy of remineralization efficacy with laser N-HAP as the most effective intervention as in Table-3.

(I) groups	(J) groups	Mean Difference	p-value	(I) groups	(J) groups	Mean Difference	p-value
CONTROL	CPP-ACP	40.1100	<0.05*	CONTROL	CPP-ACP	-106.7800	<0.05*
	N-HAP	48.0100	<0.05*		N-HAP	-162.0600	<0.05*
	ZN-HAP	37.5000	<0.05*		ZN-HAP	-148.7700	<0.05*
	LASER CPP-ACP	29.6200	<0.05*		LASER CPP-ACP	-161.1600	<0.05*
	LASER N-HAP	30.8500	<0.05*		LASER N-HAP	-203.5900	<0.05*
	LASER ZN-HAP	48.6800	<0.05*		LASER ZN-HAP	-181.9200	<0.05*
CPP-ACP	CONTROL	-40.1100	<0.05*	CPP-ACP	CONTROL	106.7800	<0.05*
	N-HAP	7.9000	<0.05*		N-HAP	-55.2800	<0.05*
	ZN-HAP	-2.6100	<0.05*		ZN-HAP	-41.9900	<0.05*
	LASER CPP-ACP	-10.4900	<0.05*		LASER CPP-ACP	-54.3800	<0.05*



	LASER N-HAP	-9.2600	<0.05*		LASER N-HAP	-96.8100	<0.05*
	LASER ZN-HAP	8.5700	<0.05*		LASER ZN-HAP	-75.1400	<0.05*
N-HAP	CONTROL	-48.0100	<0.05*	N-HAP	CONTROL	162.0600	<0.05*
	CPP-ACP	-7.9000	<0.05*	CPP-ACP	55.2800	<0.05*	
	ZN-HAP	-10.5100	<0.05*	ZN-HAP	13.2900	<0.05*	
	LASER CPP-ACP	-18.3900	<0.05*	LASER CPP-ACP	.9000	<0.05*	
	LASER N-HAP	-17.1600	<0.05*	LASER N-HAP	-41.5300	<0.05*	
	LASER ZN-HAP	.6700	<0.05*	LASER ZN-HAP	-19.8600	<0.05*	
ZN-HAP	CONTROL	-37.5000	<0.05*	ZN-HAP	CONTROL	148.7700	<0.05*
	CPP-ACP	2.6100	<0.05*	CPP-ACP	41.9900	<0.05*	
	N-HAP	10.5100	<0.05*	N-HAP	-13.2900	<0.05*	
	LASER CPP-ACP	-7.8800	<0.05*	LASER CPP-ACP	-12.3900	<0.05*	
	LASER N-HAP	-6.6500	<0.05*	LASER N-HAP	-54.8200	<0.05*	
	LASER ZN-HAP	11.1800	<0.05*	LASER ZN-HAP	-33.1500	<0.05*	
LASER CPP-ACP	CONTROL	-29.6200	<0.05*	LASER CPP-ACP	CONTROL	161.1600	<0.05*
	CPP-ACP	10.4900	<0.05*	CPP-ACP	54.3800	<0.05*	
	N-HAP	18.3900	<0.05*	N-HAP	-.9000	<0.05*	
	ZN-HAP	7.8800	<0.05*	ZN-HAP	12.3900	<0.05*	
	LASER N-HAP	1.2300	<0.05*	LASER N-HAP	-42.4300	<0.05*	
	LASER ZN-HAP	19.0600	<0.05*	LASER ZN-HAP	-20.7600	<0.05*	
LASER N-HAP	CONTROL	-30.8500	<0.05*	LASER N-HAP	CONTROL	203.5900	<0.05*
	CPP-ACP	9.2600	<0.05*	CPP-ACP	96.8100	<0.05*	
	N-HAP	17.1600	<0.05*	N-HAP	41.5300	<0.05*	
	ZN-HAP	6.6500	<0.05*	ZN-HAP	54.8200	<0.05*	
	LASER CPP-ACP	-1.2300	<0.05*	LASER CPP-ACP	42.4300	<0.05*	
	LASER ZN-HAP	17.8300	<0.05*	LASER ZN-HAP	21.6700	<0.05*	
LASER ZN-HAP	CONTROL	-48.6800	<0.05*	LASER ZN-HAP	CONTROL	181.9200	<0.05*
	CPP-ACP	-8.5700	<0.05*	CPP-ACP	75.1400	<0.05*	
	N-HAP	-.6700	<0.05*	N-HAP	19.8600	<0.05*	
	ZN-HAP	-11.1800	<0.05*	ZN-HAP	33.1500	<0.05*	
	LASER CPP-ACP	-19.0600	<0.05*	LASER CPP-ACP	20.7600	<0.05*	
	LASER N-HAP	-17.8300	<0.05*	LASER N-HAP	-21.6700	<0.05*	

Table 3: showing pre and post treatment phases in blue and red columns respectively



Treatment efficacy comparison baseline v/s final

Treatment Group	Absolute Reduction	Percentage Reduction
Control	283.5	69.8%
CPP-ACP	136.6	37.3%
N-HAP	73.4	20.5%
ZN-HAP	97.2	26.4%
CPP-ACP + Laser	92.7	24.6%
N-HAP + Laser	49.0	13.1%
ZN-HAP + Laser	52.9	14.8%

5. DISCUSSION

Dental erosion remains a growing concern in contemporary dentistry, largely driven by the widespread consumption of carbonated beverages and other acidic dietary products. Unlike caries, which is primarily bacterial in origin, erosion represents a chemical dissolution of enamel and dentin without microbial involvement^[1]. This distinction underscores the need for preventive strategies that go beyond conventional fluoride-based approaches, especially given the limitations of fluoride in penetrating subsurface lesions and its potential adverse effects at higher concentrations. In

this context, biomimetic remineralising agents such as casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), nano-hydroxyapatite (n-HAP), and zinc-substituted hydroxyapatite (Zn-HAP), as well as adjunctive technologies like Er,Cr:YSGG laser irradiation, have emerged as promising alternatives^[3,4,8]. The present study sought to evaluate the comparative remineralisation potential of these agents, both alone and in combination with laser therapy, using microhardness testing and confocal microscopy.^[8,9]

The results clearly demonstrated that all intervention groups provided significant protection



against erosive demineralisation compared to the untreated control. The control specimens exhibited a dramatic reduction in Vickers microhardness, with nearly 70% loss of surface hardness, confirming the destructive potential of carbonated beverages on enamel. In contrast, the biomimetic agents mitigated hardness loss to varying degrees, with CPP-ACP showing the least protective effect, while n-HAP and Zn-HAP performed better. Importantly, the addition of Er,Cr:YSGG laser irradiation consistently enhanced the remineralisation outcomes across all agents, with the n-HAP + laser group achieving the highest final hardness values and the smallest percentage reduction. These findings highlight both the intrinsic efficacy of biomimetic materials and the synergistic role of laser therapy in promoting enamel repair.^[4,8]

The superior performance of nano-hydroxyapatite, particularly when combined with laser irradiation, can be attributed to its unique physicochemical properties. Owing to its nanoscale dimensions and chemical similarity to natural enamel, n-HAP can penetrate enamel micropores and subsurface lesions, acting as a reservoir of calcium and phosphate ions. This deeper infiltration contrasts with fluoride, which primarily acts at the surface level. The laser irradiation likely enhanced this effect by creating microspaces within the enamel, facilitating ion entrapment and diffusion. Moreover, Er,Cr:YSGG laser treatment induces morphological and chemical changes in enamel, such as increased crystallinity and reduced carbonate content, which improve acid resistance. Together, these mechanisms explain the remarkable remineralisation depth and hardness retention observed in the n-HAP + laser group.^[13,14]

Zinc-substituted hydroxyapatite also demonstrated promising results, particularly when combined with laser therapy. Zn-HAP microparticles are biomimetic to natural enamel and dentin minerals, and their ability to adhere to enamel surfaces allows

them to form a sacrificial protective layer. Zinc ions additionally contribute antimicrobial properties and may stabilize the hydroxyapatite lattice, further enhancing remineralisation. In this study, Zn-HAP + laser achieved the second-highest hardness values, suggesting that the combination of biomimetic mineral supplementation and laser-induced microstructural modifications provides a robust protective effect. Interestingly, Zn-HAP alone performed better than CPP-ACP, underscoring its potential as a fluoride-independent preventive agent.^[3,5]

CPP-ACP, long considered the gold standard among non-fluoride remineralising agents, showed substantial efficacy but was outperformed by both n-HAP and Zn-HAP in this study. CPP stabilizes amorphous calcium phosphate clusters, maintaining a state of supersaturation at the tooth surface and thereby promoting remineralisation. However, its effect appears more surface-limited compared to the deeper penetration capacity of n-HAP. The addition of laser irradiation improved CPP-ACP outcomes, but even then, its performance lagged behind the other biomimetic agents. This finding aligns with recent literature suggesting that while CPP-ACP remains effective, newer biomimetic materials may offer superior benefits in managing erosive lesions.^[3,13]

The confocal microscopy analysis further corroborated the microhardness findings, revealing greater remineralisation depth in laser-assisted groups compared to non-laser groups.^[9] Notably, Zn-HAP with laser showed the most uniform remineralisation pattern, suggesting that its microparticles, aided by laser-induced microspaces, were able to integrate more evenly into the enamel structure. This uniformity may have important clinical implications, as consistent mineral deposition across the lesion depth is critical for long-term stability and resistance to future acid challenges.



From a clinical perspective, these findings support the integration of biomimetic remineralising agents, particularly n-HAP and Zn-HAP, into preventive and therapeutic protocols for early erosive lesions. The adjunctive use of Er,Cr:YSGG laser appears to significantly enhance the efficacy of these agents, offering a conservative, minimally invasive approach to enamel repair. Such strategies are especially valuable in populations with high consumption of acidic beverages, where erosion risk is elevated. Moreover, the fluoride-independent nature of these interventions addresses concerns regarding fluoride overexposure, making them suitable for patients seeking alternative preventive options.^[7,11]

Nevertheless, certain limitations must be acknowledged. The study was conducted *in vitro*, and while the experimental design simulated erosive cycles, the oral environment is more complex, involving factors such as salivary flow, pellicle formation, and mechanical wear. Clinical trials are therefore necessary to validate these findings *in vivo*. Additionally, the long-term durability of laser-assisted remineralisation remains to be established, particularly under repeated acid challenges and mechanical stresses. Future research should also explore the optimal laser parameters and application protocols to maximize efficacy while ensuring safety.

In conclusion, this study provides compelling evidence that biomimetic remineralising agents, particularly nano-hydroxyapatite and zinc-substituted hydroxyapatite, significantly enhance enamel resistance against erosion, and that Er,Cr:YSGG laser irradiation further amplifies these effects. The combination of n-HAP and laser therapy emerged as the most effective intervention, achieving superior hardness retention and remineralisation depth. These findings highlight the potential of laser-assisted biomimetic remineralisation as a conservative, fluoride-independent approach for managing early erosive

lesions, paving the way for innovative preventive strategies in modern dentistry.

6. CONCLUSION

- Use of Er, Cr, YSGG LASER showed significant increase in microhardness compared to Non-LASER groups, N-HAP & Zn-HAP with LASER groups showed highest values than CPP-ACP, With no statistical significant difference between N-HAP & Zn-HAP groups either with or without laser
- Use of Er, Cr, YSGG LASER showed significant increased depth of remineralisation compared to Non-LASER groups Zn-HAP + LASER [Biorepair Plus] showed more uniform highest depth of remineralisation than other groups

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