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Comparative Evaluation oof Carnosic Acid, Calcium Hydroxide and Triple Antibiotic Paste on the Microhardness of Radicular Dentin– An in Vitro Study

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Introduction- *Carnosic acid, a plant-based compound, exhibits a broad spectrum of biological activities, including potent antioxidant, anti-inflammatory, antibacterial, and anticancer properties. In endodontic therapy, various intracanal medications are employed to reduce microbial loads within the root canal system. However, understanding the impact of these medications on the pulp-dentine complex is crucial before clinical application. This study investigates the effects of carnosic acid on radicular dentine, comparing its outcomes with those of established intracanal medications, calcium hydroxide and triple antibiotic paste.*

AIM- *This study aimed to evaluate and compare the effects of carnosic acid, calcium hydroxide, and triple antibiotic paste on the microhardness of radicular dentin.*

Material's & Method- This study utilized 50 extracted mandibular bicuspids, which underwent initial canal negotiation and biomechanical preparation with ProTaper Gold files. The roots were divided into five groups (n = 10 each): an untreated control, a biomechanically prepared control, and three groups receiving intracanal medicaments (triple antibiotic paste, calcium hydroxide, or carnosic acid). After 21 days, medicaments were removed, and Vickers microhardness testing was conducted to evaluate the effects of each medicament on root dentin microhardness.

Result- *A comparative analysis of the three intracanal medications showed a significant decline in microhardness, with carnosic acid causing the greatest reduction, followed by triple antibiotic paste. Calcium hydroxide had the least impact on microhardness.*

Conclusion- *The results showed that 21-day exposure to the tested intracanal medications significantly reduced radicular dentine microhardness. Carnosic acid, in particular, merits further investigation due to its promising antibacterial properties and potential as an effective intracanal medicament.*

Keywords- *Carnosic Acid, Calcium Hydroxide, Triple Antibiotic Paste, Vickers microhardness testing*

INTRODUCTION-

Root canal infections are primarily driven by complex microbial communities, whose development and persistence are challenging to anticipate and quantify. To effectively combat these infections, it is crucial to identify the key physiological and ecological factors that influence microbial colonization, including resilience (the ability to recover from disruptions) and resistance (tolerance to disturbances). Notably, Gram-positive bacteria, particularly Enterococcus faecalis, have been frequently isolated from failed endodontic treatments, highlighting their role in persistent root canal infections.[1]

Enterococcus faecalis, a versatile facultative anaerobic Gram-positive bacterium, plays a pivotal role in the development of root canal infections. This resilient microorganism can thrive under extreme conditions, including alkaline pH, elevated temperatures, nutrient scarcity, and exposure to surfactants.[2] E. faecalis can infiltrate dentinal tubules [3] rendering endodontic instrumentation and irrigation ineffective during chemo mechanical preparation. Furthermore, it exhibits both intrinsic and extrinsic resistance to multiple antimicrobial agents, as evidenced by numerous studies.[4,5] Its ability to form biofilms within root canals also enables persistence despite intracanal antimicrobial treatments.[2]

The eradication of Enterococcus faecalis biofilms from infected root canals poses a significant challenge. As a result, various intracanal medications have been explored, including calcium hydroxide-based pastes, chlorhexidine, steroids, and antibiotic combinations.[6] Additionally, plant-based intracanal medicaments have been investigated as potential alternatives, highlighting the ongoing search for effective solutions to combat E. faecalis biofilms in root canal infections.

Calcium hydroxide [Ca(OH)2]-based paste has been a cornerstone in endodontic therapy for nearly a century, with its use dating back to Herman's pioneering work in 1920. The antimicrobial properties of Ca(OH)2 can be attributed to its high alkaline pH, which disrupts bacterial cell membranes and compromises microbial DNA integrity. Furthermore, Ca(OH)2 has been shown to promote the formation of hard tissue-like structures by upregulating alkaline phosphatase activity, highlighting its potential in pulp capping and intracanal medicament applications.[7]

The triple antibiotic paste (TAP), comprising metronidazole, ciprofloxacin, and minocycline, has demonstrated efficacy in endodontic therapy. However, despite its promising outcomes, TAP has been associated with an undesirable side effect: discoloration of the coronal tooth structure.[8]

Certain plant extracts, renowned for their antimicrobial properties, have been harnessed for medicinal applications. Rosmarinus officinalis L., a member of the Lamiaceae family, is a versatile, edible evergreen shrub that boasts an impressive array of bioactive properties, including antioxidant, anti-inflammatory, anticancer, and antimicrobial activities. Key bioactive compounds isolated from this plant include rosmarinic acid, carnosic acid, carnosol, ursolic acid, oleanolic acid, genkwanin, apigenin, and luteolin, which contribute to its therapeutic and culinary value.[9] An in vitro investigation assessed the antimicrobial efficacy of commercial rosemary extract formulations against selected bacterial strains. The results indicated that Gram-positive bacteria exhibited greater susceptibility to the rosemary extracts, particularly those containing carnosic acid in oil-soluble formulations.[10] Given the limited research on the use of carnosic acid as an intracanal medicament, this in vitro study aimed to investigate its effects, in comparison to calcium hydroxide and triple antibiotic paste, on the microhardness of radicular dentin.

MATERIALS AND METHODS-

1. Selection parameters for test samples- This study utilized a sample of 50 freshly extracted mandibular bicuspid teeth, selected based on specific criteria. Teeth with straight, single canals were included, while those with cavities, fractures, curved or calcified canals were excluded to ensure consistency and accuracy in the investigation.

To ensure sample integrity, each tooth was verified to be free from debris and calculus. Furthermore, all samples underwent ultrasonic cleaning with a 5.25% sodium hypochlorite solution prior to the start of the investigation.

2. Preparation of test samples - The crown structure of each tooth was removed at the cementoenamel junction using a diamond bur, exposing the root. The root surface was then flattened to create a uniform surface perpendicular to the long axis of the tooth.

ProTaper rotary gold files with F3 size (Dentsply, Maillefer) were utilized for root canal instrumentation. Each time the instrument was changed, the canals were irrigated with 5 mL of a 17% EDTA solution(Safe Endo smart prep liquid, India). This was followed by the addition of 5 mL of a 5.25% sodium hypochlorite solution(Neelkanth, India), and the final irrigation was performed using 5 mL of a hypotonic normal saline solution.

3. Categorization of test samples - The specimens were divided into four groups based on the medicament used. A control group of 20 teeth remained untreated. Group A consisted of 10 specimens, where a mixture of carnosic acid powder (Sigma Aldrich) and saline (1:1 wt./vol) was placed in the root canal. Group B comprised 10 samples treated with a triple antibiotic paste, prepared by mixing ciprofloxacin, metronidazole, and minocycline (1:1:1 ratio) with saline solution.

Group C consisted of 10 specimens, where calcium hydroxide powder(Neelkanth Ortho Dent LTD, India) was blended with saline solution at a 1.5:1 weight-to-volume ratio and inserted into the root canal. The control group, denoted as Group D, comprised 20 samples, divided into two subsets: one subset of 10 samples underwent immediate testing, while the remaining 10 samples were evaluated after a 14-day period, concurrently with the other experimental groups.
4. Intracanal Delivery Of Therapeutic Agents -All intracanal medicaments, in either paste or gel form, were carefully placed within the canal spaces, ensuring they reached the apical foramen with the assistance of a lentulospiral. The root canal access was then temporarily sealed using a cotton pellet and Cavit ((ESPE, Seefeld, Germany). The treated teeth were subsequently incubated at 37°C and 100% humidity for 14 days to mimic clinical conditions. After this period, the apices of the treated teeth were sealed with heated glue.
5. Clearance of root canal medicaments-Following the 14-day incubation period, the intracanal medicaments were thoroughly removed from the root canal system using a combination of sodium hypochlorite irrigation solution and manual instrumentation with H-files and rotary endodontic files. To eliminate any remaining sodium hypochlorite, all samples underwent a final irrigation with 2 mL of distilled water. Paper points were then used to dry the root canals. The canals were subsequently sectioned into 2-mm thick transverse segments using a diamond-coated disc. To provide stability, the roots were embedded in resin blocks. Initial microhardness measurements were taken using a microhardness tester fitted with a Knoop diamond indenter.
6. Investigation of microhardness changes in experimental samples-Three consecutive Knoop hardness indentations were made at standardized locations on the pulpal surface, approximately 1 mm distant from the root canal. The mean Knoop hardness number (KHN) was then calculated for each specimen. To ensure consistency, a single investigator performed all procedures, including disinfection with sodium hypochlorite and preparation of acrylic resin blocks. Subsequent microhardness testing was conducted by a second investigator, who remained blinded to the specific intracanal medicament used for each specimen.

RESULT-

Knoop microhardness testing evaluated root dentin microhardness. One-way ANOVA and post hoc analysis (Table 1) revealed significant differences among groups, with microhardness decreasing in the following order: control > calcium hydroxide > triple antibiotic paste > carnosic acid.
The mean hardness values recorded after 14 days are presented in Table 2. Significant differences were observed among the groups ($p \leq 0.005$). The control group exhibited the highest mean hardness (45.72 ± 8.30), followed by the Calcium hydroxide group (35.9350 ± 4.98479), the Triple antibiotic paste group (31.6030 ± 7.50516), and the Carnosic acid group (18.4120 ± 3.49025).
Table 3: Intra-group Comparison: This table shows how the hardness of different intra-canal medications varies within a group. Carnosic acid - Triple antibiotic paste, Carnosic acid - Calcium hydroxide, Carnosic acid - Control group, Triple antibiotic paste - Calcium hydroxide, and Calcium hydroxide - Control group all had significantly different mean hardness values, according to statistical analysis ($p \leq 0.005$). The Triple Antibiotic Paste-Control group pair did not, however, vary statistically significantly ($p \geq 0.005$).

DISCUSSION-

The majority of organisms and their biofilms can be eliminated by chemo mechanically preparing the canal system, but some chronic organisms—particularly E. faecalis—in the root canal are difficult to remove.[11] Enterococcus faecalis (E. faecalis), a facultative anaerobe, was chosen for this study due to its high prevalence in secondary endodontic infections. Eradicating E. faecalis poses a significant challenge due to its remarkable resilience, which enables it to withstand in Starvation, High pH levels, High salt concentrations, Biofilm formation, Spore formation, Antibiotic resistance. Furthermore, under in vivo conditions, the molecular-level pathogenesis of enterococcal infections is attributed to oxidative stress caused by the production of free radicals, such as superoxide and hydrogen peroxide.[12]
In vitro studies have shown that prolonged exposure to calcium hydroxide compromises the mechanical properties of radicular dentin. In contrast, herbal intracanal medicaments offer a promising alternative, as they do not adversely affect dentin's mechanical properties while exhibiting comparable antimicrobial efficacy.[13] Carnosic acid, a bioactive compound derived from herbal sources, exhibits a multifaceted therapeutic profile. Its antioxidative properties effectively mitigate oxidative stress and cellular damage

induced by free radicals. Additionally, carnosic acid demonstrates antimicrobial activity, anti-inflammatory effects, and a favourable toxicity profile, rendering it a promising intracanal medicament.[14]
The carnosic acid group in this study exhibited a low proportion of living and dead bacteria, indicating its high antibacterial efficacy, potentially inhibiting bacterial proliferation. While the exact mechanism of carnosic acid remains unknown, its lipophilic nature allows it to readily penetrate bacterial cell walls. Carnosic acid is believed to modulate membrane permeability and interfere with the ethidium bromide efflux mechanism.[15] additionally, carnosic acid has been found to suppress the activation of genes associated with pathogenicity and biofilm formation, making it a promising quorum sensing antagonist against staphylococcus aureus. At the molecular level, carnosic acid's antioxidant properties may inhibit the generation of free radicals, crucial for the survival of enterococci, by scavenging them.[16]
Carnosic acid's high purity and low ph (ranging from 2.0 to 2.5) can lead to significant demineralization of radicular dentin, resulting in the greatest reduction in microhardness among all groups.[17] due to its higher organic content (primarily collagen)[18] and lower mineral composition compared to enamel, dentin is more susceptible to acid erosion. Acids with a low ph and high titratable acidity (the ability to maintain an acidic environment) can lead to:

- **Surface softening:** the loss of hydroxyapatite crystals weakens the structural integrity of dentin.
- **Collagen degradation:** acid exposure can denature collagen fibres, further compromising dentin strength.[19]
- **Increased permeability:** as dentin erodes, its porosity increases, making it more prone to cavities and sensitivity.[20]

Triple antibiotic paste (tap) is widely used to disinfect root canals in cases of persistent infections and during regenerative endodontic procedures. However, its extended application can weaken the structural integrity of radicular dentin by markedly lowering its microhardness. This adverse effect is mainly due to tap's chelating action, which breaks down the organic collagen framework of the dentin while leaving the inorganic hydroxyapatite largely unaffected.[21,22]
While ca(oh)2 is widely used as an intracanal medication, it resulted in a moderate to intermediate decrease in root dentin microhardness. This damage may be caused by the highly alkaline nature of ca(oh)2 (ph 11.8), leading to disintegration of the dentin structure or destruction of the organic matrix. Additionally, infiltration into the intrafibrillar framework of mineralized collagen fibrils by ca(oh)2 molecules may alter the 3-d confirmation of tropocollagen and reduce dentin microhardness.[23,24] research indicates that calcium hydroxide requires an exposure period of more than four weeks to produce a significant reduction in dentin microhardness. Consequently, when applied for shorter durations, the decrease in microhardness is relatively minimal.
Mechanical instrumentation removes a small layer of dentin, and this slight loss is associated with a reduction in the tooth's microhardness. The process of preparing the root canal by shaving off dentin alters its microstructure, which in turn results in a modest decrease in hardness. Within the constraints of this study, no research has yet examined how carnosic acid influences the microhardness of radicular dentin. Consequently, additional investigations are necessary to determine the clinical implications and potential benefits of using carnosic acid.

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REFERENCES-

1.Hancock HH 3rd, Sigurdsson A, Trope M, Moiseiwitsch J. Bacteria isolated after unsuccessful endodontic treatment in a North American population. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001;91:579-86.
2.Hamed SA, Shabayek S, Hassan HY. Biofilm elimination from infected root canals using four different single files. BMC Oral Health 2022;22:660.
3. Siqueira JF Jr., de Uzeda M, Fonseca ME. A scanning electron microscopic evaluation of in vitro dentinal tubules penetration by selected anaerobic bacteria. J Endod 1996;22:308-10.
4. Siqueira JF Jr., de Uzeda M. Disinfection by calcium hydroxide pastes of dentinal tubules infected with two obligate and one facultative anaerobic Bacteria. J Endod 1996;22:674-6.
5. Haapasalo M, Orstavik D. In vitro infection and disinfection of dentinal tubules. J Dent Res 1987;66:1375-9.
6.Tilakchand M, Hegde S, Naik B. Evaluation of the efficacy of a novel antibiotic-steroid paste versus conventionally used intracanal antibiotic pastes and irrigating solutions against a 3-week-old biofilm of Enterococcus faecalis. J Conserv Dent 2020;23:436-40.
7.Mohammadi Z, Dummer PM. Properties and applications of calcium hydroxide in endodontics and dental traumatology. Int Endod J 2011;44:697-730.

8.Kim JH, Kim Y, Shin SJ, Park JW, Jung IY. Tooth discoloration of immature permanent incisor associated with triple antibiotic therapy: A case report. J Endod 2010;36:1086-91.
9. Bernardes WA, Lucarini R, Tozatti MG, Souza MG, Silva ML, Filho AA, et al. Antimicrobial activity of Rosmarinus officinalis against oral pathogens: Relevance of carnosic acid and carnosol. Chem Biodivers. 2010;7:1835–40.
10. Klancnik A, Guzej B, Kolar MH, Abramovic H, Mozina SS. In vitro antimicrobial and antioxidant activity of commercial rosemary extract formulations. J Food Prot. 2009;72:1744–52.
11. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: Its role in root canal treatment failure and current concepts in retreatment. J Endod. 2006;32:93–8.
12. Szemes T, Vlkova B, Minarik G, Tothova L, Drahovska H, Turna J, et al. On the origin of reactive oxygen species and antioxidative mechanisms in Enterococcus faecalis. Redox Rep 2010; 15: 202–6.
13. Naeem MM, Sarwar H, Nisar A, Ahmed S, Shabbir J, Khurshid Z, et al. Effect of propolis on root dentine microhardness when used as an intracanal medicament: An in vitro study. J Funct Biomater 2023; 14: 144.
14. Wang QL, Li H, Li XX, Cui CY, Wang R, Yu NX, et al. Acute and 30-day oral toxicity studies of administered carnosic acid. Food Chem Toxicol 2012; 50: 4348–55
15. . Vaara M. Agents that increase the permeability of the outer membrane. Microbiol Rev. 1992;56:395–411
16. . Nakagawa S, Hillebrand GG, Nunez G. Rosmarinus off icinalis L.(Rosemary) extracts containing carnosic acid and carnosol are potent quorum sensing inhibitors of Staphylococcus aureus virulence. Antibiotics(Basel) 2020;9:149
17. David T. BaileySteven L. RichheimerVirginia R. BankBenjamin T. King.High purity carnosic acid from rosemary and sage extracts by pH-controlled precipitation.United States patent US5859293A
18. West NX, Hughes JA, Addy M. The effect of pH on the erosion of dentine and enamel by dietary acids in vitro. J Oral Rehabil. 2001 Sep;28(9):860-4.
19. Fernández CE, Brandao ACS, Bícego-Pereira EC, Del Bel Cury AA, Cury JA, Tenuta LMA. Effect of pH and titratable acidity on enamel and dentine erosion. Clin Oral Investig. 2022 Sep;26(9):5867-5873.
20. Fernández, C.E., Brandao, A.C.S., Bícego-Pereira, E.C. et al. Effect of pH and titratable acidity on enamel and dentine erosion. Clin Oral Invest 26, 5867–5873 (2022).
21. Gúzman S, Caccia M, Cortés O, Bolarin JM, Requena A, Garcia-Godoy A, Garcia-Godoy F, Boj JR. Human root dentin microhardness and degradation by triple antibiotic paste and calcium hydroxide. Am J Dent. 2022 Aug;35(4):205-211
22. Prather BT, Ehrlich Y, Spolnik K, Platt JA, Yassen GH. Effects of two combinations of triple antibiotic paste used in endodontic regeneration on root microhardness and chemical structure of radicular dentine. J Oral Sci. 2014 Dec;56(4):245-51.
23. Twati, W.A., Wood, D.J., Liskiewicz, T.W. et al. An Evaluation of the Effect of Non-Setting Calcium Hydroxide on Human Dentine: A pilot Study. Eur Arch Paediatr Dent 10, 104–109 (2009).
24. Al-Hiyasat AS, El-Farraj HS, Alebrahim MA. The effect of calcium hydroxide on dentine composition and root fracture resistance of human teeth: An in vitro study. Eur J Oral Sci. 2021 Aug;129(4):e12798

List Of Table-

Intra-canal medicament	N	Minimum	Maximum	Mean	Std. Deviation
Carnosic acid	10	13.02	23.93	18.4120	3.49025
Triple antibiotic paste	10	18.98	42.39	31.6030	7.50516
Calcium hydroxide	10	28.32	44.39	35.9350	4.98479
Control	10	43.93	63.09	54.1220	6.13477

Table 1 illustrates the microhardness levels among various intra-canal medicaments.

Intra-canal medicament	N	Mean	Std. Deviation	f-value	p-value
Carnosic acid	10	18.4120	3.49025	31.09	0.001 (s)
Triple antibiotic paste	10	31.6030	7.50516		
Calcium hydroxide	10	35.9350	4.98479		
Control	10	45.72	8.30		

Table 2: mean comparison of micro-hardness of all groups with baseline:
(Statistical test: Paired t-test; ($p<0.05$- significant, CI=95%), N= number of study subjects, s- significant, n.s –not significant.)

Group	Group	Mean diff	p-value
Carnosic acid	Triple antibiotic paste	-13.19100	0.001 (s)

	Calcium hydroxide	-17.52300	0.001 (s)
	Control	-27.31000	0.001 (s)
Triple antibiotic paste	Calcium hydroxide	-4.33200	0.436 (n.s)
	Control	-14.11900	0.001 (s)
Calcium hydroxide	Control	-9.78700	0.008 (s)

Table 3: Intra-group Comparison -This table illustrates the intra-group comparison of hardness among various intra-canal medicaments
(Statistical test: Tukey's Post Hoc test; (p<0.05- significant, CI=95%), N= number of study subjects, s-significant, n.s –not significant.)