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ORIGINAL ARTICLE

Triple antibiotic paste versus nano calcium hydroxide as an intracanal medicament in human primary molars: a randomized clinical trial

ABSTRACT

Aim: *Intracanal medicaments play a critical role in cleaning microbial biofilm, dentin tubule sterilization, and successful endodontic treatment. This study aimed to evaluate the antibacterial effect of Nano-calcium hydroxide (NCH) and Triple Antibiotic Paste (TAP) as intra-canal medicaments in human non-vital primary molar teeth.*
Methodology: *The NCH particles were characterized by transmission electron microscopy (TEM), Fourier transform infrared (FTIR) spectroscopy, and X-ray diffraction (XRD). This study included 39 primary molar teeth with an indication for non-vital pulp therapy. Following the access cavity preparation, a microbial sample (S_1) of each tooth was collected and canals were prepared by chemo-mechanical technique. Using dynamic block randomization, canals were divided into three groups based on the type of medicament. The second microbial sample (S_2) was collected 7 days after ICM application. The canal's aerobic and anaerobic micro-organisms load was calculated by counting colony-forming units (CFUs).*
Results*: The TEM, FTIR, and XRD characterization techniques confirm the NCH nanoparticle formation. NCH showed a reduction of the number of aerobic and anaerobic micro-organisms by 98.09% and 90.79%, respectively. While TAP had greater antibacterial activity compared to NCH aerobic (99.95%) and anaerobic (99.78%) micro-organisms. NCH and TAP showed a statistically significant difference (P<0.005) in bacterial elimination of the root canals in comparison with chemo-mechanical irrigation alone.*
Conclusion: *Both TAP and NCH antibacterial activity were approved during the endodontic.*

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Introduction

Primary teeth play a critical role in maintaining proper occlusion and the development of the oral cavity (1). The invasion of microorganisms and their toxins, which are mainly bacterial products, into dental tissue is a common clinical problem in primary teeth (2). Infectious may lead to inflammation, abscesses, cysts, apical granulomas, dissolution of tooth tissue, oral lesions, and even osteomyelitis (3, 4). Retaining primary teeth requires successful endodontic treatment that relies on cleaning and disinfecting microbial biofilm in the root canal system and preventing the proliferation and reentry of bacteria in the root canal system (5, 6).

Among the various kinds of intracanal medicaments, the triple antibiotic paste (TAP) and conventional calcium hydroxide (CH) are commonly used intracanal medicaments for endodontic treatment. One such medication is TAP, which contains three antibiotics including permanent metronidazole, ciprofloxacin, and minocycline. TAP can be successfully applied to disinfect canals and treat periapical pathology. However, this approach's restrictions are the development of antimicrobial resistance and tooth discoloration (7-9). CH ($Ca(OH)_2$) with a size range from 1 to 10 μm, is the "Gold Standard" endodontic medicament to control residual bacteria in the prepared canal that has strong antimicrobial activity. CH damages the bacterial cytoplasmic membrane and DNA. However, some microorganisms resist to CH (10). To overcome this, minimizing the CH particle diameter and forming CH nanoparticles may have been sought to improve the eradication of bacteria. Nano-calcium hydroxide (NCH) should possess a higher surface-to-volume ratio with their greater interaction in dentinal tubules which results in remarkable improvement in antibacterial and antifungal properties, long-lasting effects, deep tissue penetration, and effective prevention of coronal microleakage (11, 12).

Numerous in vitro and clinical studies have assessed the efficacy of different intracanal medicaments in reducing bacterial infections in teeth. Calcium hydroxide (CH) has been found to reduce bacterial colony counts, particularly *Enterococcus faecalis* (*E.faecalis*) when used alone or in combination with chlorhexidine or TAP (13, 14). TAP and CH have been shown to improve the development of pulp dentin complex and disinfect immature teeth (15). Various studies compared the antimicrobial efficacy of TAP and CH. In a study TAP showed a slightly stronger antimicrobial efficacy against *E. faecalis*, compared to CH; however, the difference wasn't statistically significant (16). Another study showed that both TAP and CH reduced initial intracanal bacterial loads, and exhibited similar levels of bacterial reduction (17). However, there are studies suggesting that TAP compared to CH had a significantly better effect on eliminating *E. faecalis* from dentinal tubules and decreasing bacterial biofilm formation (18-20). Furthermore, in a previous study, the group treated with NCH showed superior antimicrobial activity and penetration of dentinal tubules versus the group treated with conventional CH (21).

Despite the importance of understanding the bacterial load in primary teeth root canals before and after intracanal medicament application, there is currently limited literature on this topic. This study aimed to address a gap in current research by focusing on the antimicrobial effectiveness of TAP and NCH in primary teeth. While prior studies have examined the antimicrobial properties of TAP and CH, most have focused on permanent teeth. Additionally, our study employed a nano-sized calcium hydroxide (NCH) formulation to explore the potential benefits of nanotechnology in dental materials. Therefore, this study aimed to assess the total count of aerobic and anaerobic bacteria in primary teeth root canals that require non-vital pulp therapy, both before and after the application of NCH and TAP intra-canal medicaments.

Materials and Methods

Study design

This randomized double-blinded controlled trial aimed to evaluate the efficacy of TAP and CH in decreasing the bacterial load of infected primary molars in children. This study was approved by the ethical committee of Shiraz University of Medical Sciences with the ethical code number of IR.SUMS.REC.1397.828 and clinical trial code of IRCT-20181226042132NI.

Participants

Participants with maxillary or mandibular primary molars with pulpal infection admitted to the endodontic ward of the faculty of dentistry clinic, Shiraz, Iran between December 2021 and June 2022 were included in this study. Participants (n=39) ages (4-6 years old) with infected maxillary or mandibular primary molars, considering a two-tailed significance level of 5% and 80% power. Exposure of the pulp with caries and fistula was determined in clinical diagnosis. Teeth with internal or external root resorption and excessive mobility as well as those with involvement of permanent dental follicles were excluded. Participants with a history of taking antibiotics for at least 1 week before the study were excluded. Written acquired consent was obtained from the parents of participants.

Randomization and Blinding

The patients were randomly assigned to three groups (each 13) according to a dynamic randomized block design. To reduce the influence of age-related variables, thirteen blocks each containing three participants with similar ages were created. Participants were randomly assigned to each treatment group. Randomization was performed with the help of an assistant. A random code number was assigned to each patient at the beginning of the study to maintain blinding. Participants were blinded to their group allocation. The operator who conducted and analyzed the microbial tests was completely unaware of the group allocations.

Intervention

- Intracanal medicament preparation

To prepare TAP, a combination of ciprofloxacin (Bayer plc, UK), metronidazole (Flagyl, Winthrop Pharmaceuticals, UK), and minocycline (Expanscience Laboratories, Paris, France) was mixed at a concentration of 20 mg/ml for each antibiotic. NCH was prepared following the procedure. A total of 100 mL sodium hydroxide (0.4 M) was added gently to the same volume of calcium nitrate dehydrate (0.4 M) on a magnetic stirrer at ambient temperature for an hour. Then, the precipitate was filtered and washed three times with distilled water. Afterward, the resulting white precipitate was placed in a container under ambient argon gas and the temperature was gradually increased to 60°C (at the rate of 1 °C/min) for 120 min. The final precipitate was maintained in a vacuum desiccator until used in the clinical trial.

- Characterization of nano-calcium hydroxide (NCH)

The size and morphology of NCH were accessed using transmission electron microscopy (TEM ZIESS machine, model EM10C, German). The spectral compatibility and chemical structure of NCH were evaluated by Fourier transform infrared (FT-IR) spectroscope (PerkinElmer, Spectrum RXI). The FT-IR spectrum was recorded in the range of 400–4000 cm-1using the KBR technique. The XRD pattern of the crystallinity and phase of NCH were carried out at ambient temperature using an X-ray diffractometer (PW 1710 BASED. Philips, the Netherlands) with Cu-Kα radiation at a wavelength of 1.54 Å and scanning rate of 1 step/s (scan size: 0.1°/step).

- Intracanal medicament administration and Microbial sample collection

The access cavity was prepared under local anesthesia (2% lidocaine, Darupakhsh, Tehran, Iran) with a high-speed handpiece and diamond burs. The standard endodontic procedure was performed under rubber-dam isolation. The pulp chamber was opened and the pulp tissue was carefully removed using hand instruments. Orifices of the larger-sized canal (distal in mandibular molars and palatal in maxillary molars) were extended by a Neolix orifice opener (NEOLIX, Châtres-la-Forêt, France). First microbiological samples (S1) were collected by inserting #20 sterile absorbable paper points (Ariadent

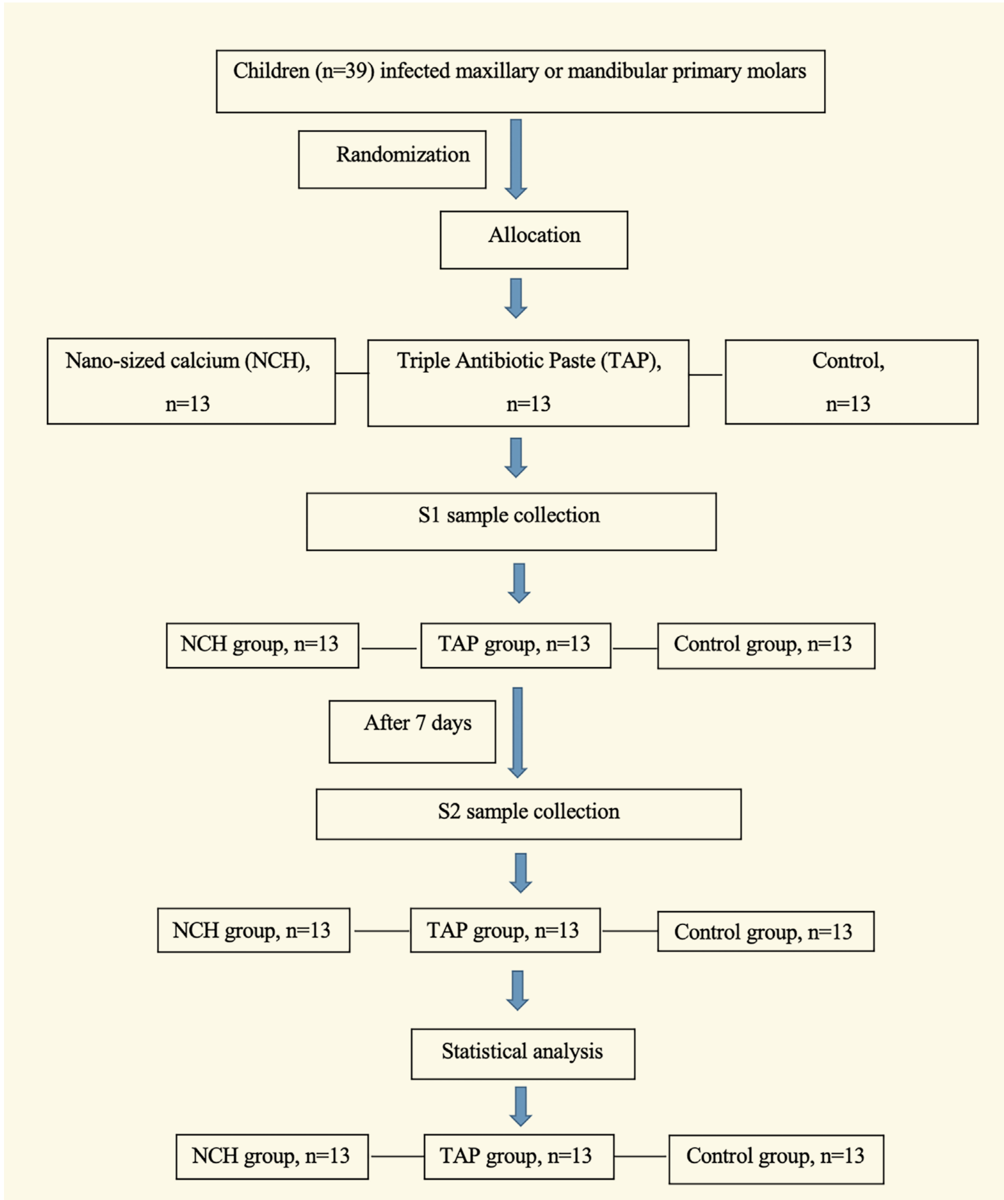


Figure 1
Diagram of the clinical procedure of the study.

Co., Tehran, Iran), after the extension of the canal, (leaving it for about 30 seconds and then transferring it into a test tube containing 2 ml brain heart infusion). The canals were then completely prepared with K- files #20,25 (Mani, Tochigi, China) and rinsed with normal saline.

After mechanical preparation, the canals were dried with sterile paper points. Subsequently, the teeth were divided into three groups in terms of receiving intracanal medicament. The TAP group received TAP, the NCH group received NCH, and the control group received rinsing with normal saline (NaCl 0.9%, Darupakhsh, Tehran, Iran).

The medicaments were applied in root canals, using lentulo spiral#25 (Dentsply Maillefer, Ballaigues, Switzerland), and the cavity was temporarily sealed with reinforced ZnOE (Kemdent, England). Seven days later, the temporary restoration was removed and the canal was irrigated with normal saline to wash out the medicaments. Second bacteriological samples (S2) were collected after the total removal of medicament residues. Then canals were obturated with Metapex (Meta Biomed Co. Ltd., South Korea), and the teeth were permanently restored with glass ionomer cement (GC Fuji IX, GC, Tokyo, Japan) followed by cementation of stainless-steel crown (3M, St. Paul, MN, USA). Figure 1 displays the diagram of the clinical procedure.

Microbiological procedures

The microbial procedure by Donyavi et al. (13) was performed in this study. A total of 1 mL of thioglycolate (for anaerobes) and 1 mL of Brain Heart Infusion (BHI, Merck, Darmstadt, Germany) broth (for aerobic bacteria) transfer mediums were used for each paper point to be immersed in and then transferred to the lab within 30 min for further analysis.

A total of 1, 2, 5, and 10 µL of each medium (thioglycolate and BHI) were collected by a sampler and immersed in 1 mL of their respective medium type. Next, 10 µL from each tube was extracted and transferred to a plate containing the mentioned medium. The collected samples from the thioglycolate and BHI medium were cultured on the Brucella agar enriched with defibrinated sheep blood and blood agar, respectively. Brucella agar plates were placed in an anaerobic jar. Using, an anaerobic environment was produced by a Gas-pak, and the jar was incubated at 37 °C for 72 h. Blood agar plates were also incubated at 37 °C for 24 h for aerobic bacteria to proliferate. Following the incubation period, plates were removed from the incubator, and formed colonies were counted by a colony counter. The mean number of colony-forming units (CFU) was reported based on the concentration of primary dilution. In addition, the percentage of reduction in the number of aerobic and anaerobic colonies was calculated for each group and reported.

Statistical analysis

Data were analyzed using SPSS software (SPSS version 26.0, SPSS, Chicago, IL, USA). Descriptive statistics were reported using mean and standard deviation. One-sample Kolmogorov-Smirnov test was used to assess the normality of the data. Kruskal-Wallis and Mann-Whitney U tests were used for group comparison. $P \leq 0.05$ was considered the statistical significance value.

Results

Nano-calcium hydroxide (NCH) characterization

An electron microscope (TEM) was used to evaluate the NCH particle's size, shape, and crystalline degree. Figure 2 (b) shows that NCH particles are randomly oriented and irregularly structured with a variable number of sides, but more resemble hexagons. The mean diameter of fabricated nanoparticles was 35.44±12 nanometers (nm), which shows the histogram of the synthesis NCH in Figure 2 (c). Moreover, many NCH with particle sizes below 40 nm and larger than 60 nm are very few.

FT-IR Spectroscopy related to the chemical structure of synthesized NCH mentioned in Figure 3 was applied to confirm the presence of both hydroxide and carbonate parts of calcium. FT-IR spectra of the NCH illustrated that the band at 3642 cm^{-1} belongs to OH stretching mode. The OH stretching absorption peak was relatively broad (3250 to 3530 cm^{-1}) and showed that hexagonal calcium hydroxide phase with a mixture of phases. The sharp 879 cm^{-1} peak is related to the ν_2 symmetric deformation, the 712 cm^{-1} peak for the ν_4 bending vibration, and the 1453 cm^{-1} is related to the ν_3 asymmetric stretching of the CO3 group. The minor peaks at 2501–2531 and 1788 cm^{-1} were due to the adsorption of the atmospheric CO2 and C=O stretching, respectively.

Figure 4 shows the XRD pattern of NCH particles. NCH showed strong peaks at 29.1, 34.2, 47, 50.5, 54.6, 62.7, 70.6, and 84° in the 2θ regions and these peaks corresponding to the (100), (101), (102), (110), (111), (200), (201), (202), and (211) planes of the hexagonal NCH phase.

Microbial tests

Children (n=39) aged 4-6 with infected maxillary or mandibular primary molars were included in the study. As shown in

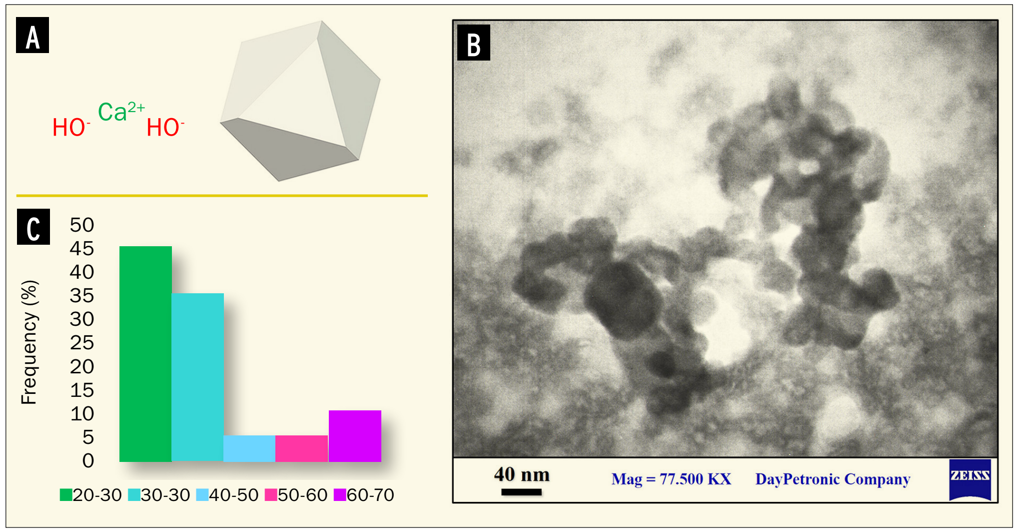


Figure 2
A) Chemical structure and schematic of calcium hydroxide nanoparticle (NCH) **B)** TEM image of NCH on the scale of 40 nm, **C)** histogram of nanoparticle diameter size distribution obtained from microstructure measurement software.

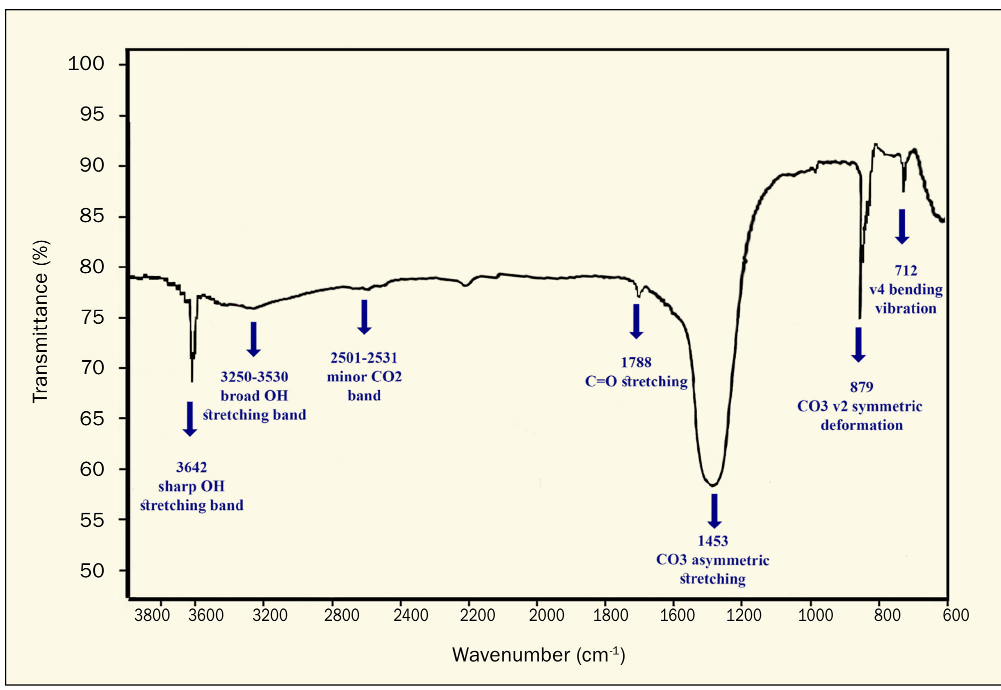


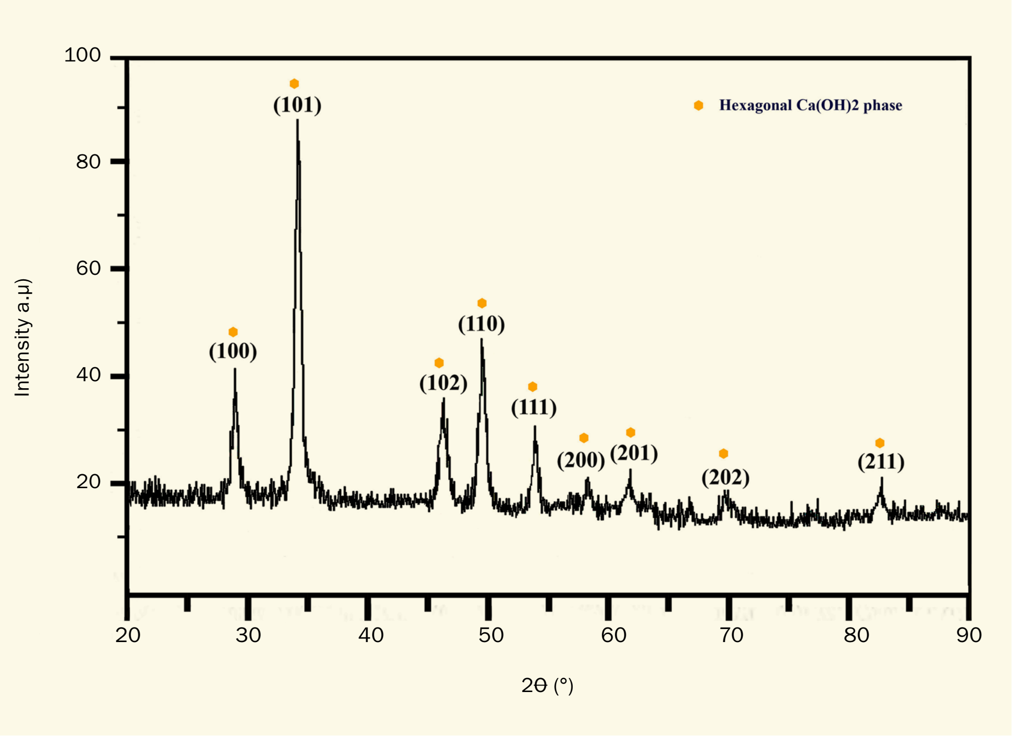
Figure 3
FT-IR spectra of NCH.

Table 1, the results demonstrated a significant reduction in the number of aerobic and anaerobic bacteria after the application of TAP (P-value<0.01) and NCH (P-value<0.01) compared to the control group. The mean reduction in the number of aerobic bacteria was 99.95% (±0.06) for TAP and 98.05% (±2.36) for NCH. The mean reduction in the number of anaerobic bacteria was 99.78% (±0.24) for TAP and 90.79% (±11.69) for NCH. The data suggests that TAP was significantly more effective than NCH in reducing the number of aerobic and anaerobic bacteria (P-value<0.01). Table 2 demonstrated that TAP had more potential in reducing the number of aerobic and anaerobic CFUs compared to NCH.

Discussion

The characterization of synthesis particles using TEM, FTIR, and XRD demonstrated the NCH formation in the present work. Moreover, the effectiveness of TAP and NCH as intracanal medicaments to reduce the number of aerobic and anaerobic mi-

Figure 4
XRD spectra of NCH.



cro-organisms in maxillary and mandibular primary molars over seven days was approved. However, our result showed that TAP had more potential in reducing the number of aerobic and anaerobic CFUs compared to NCH. As pulpal infection is a complex microbial process, evaluation of both aerobic and anaerobic bacteria in the root canals was deemed critical (22). Although chemo-mechanical debridement of the root canals reduces up to 53% of intracanal micro-organisms, the use of intracanal medicaments is essential to achieve more effective eradication of micro-organisms (14, 23, 24). This is especially crucial in primary teeth due to their ribbon-shaped canal anatomy and the presence of deeply penetrated bacteria in the dentinal tubules (14, 25).

The comparison of CFU counts before and after the application of TAP and NCH showed a significant reduction in the number of CFUs. The average aerobic bacterial CFUs before intracanal medicament application in TAP, NCH, and control groups were 4.7×10^4, 4.07×10^4, and 6.77×10^4, respectively. These measurements for anaerobic bacteria were 1.29×10^5, 4.31×10^4, and 1.02×10^5. The present study's CFU results on primary teeth align with previous studies, indicating that overall anaerobic CFU was higher than aerobic CFU and ranged between 10^2 to 10^8 (13, 26, 27). The application of NCH and TAP resulted in a significant reduction in both aerobic and anaerobic bacteria; and, TAP showed a higher reduction percentage compared to NCH. Results of the present study demonstrated a 98.09% and 90.79% significant reduction in aerobic and anaerobic bacteria, respectively, when applying NCH. In comparison, TAP showed a reduction of the number of aerobic and anaerobic micro-organisms by 99.95% and 99.78%, respectively. TAP used as intracanal medicaments, has shown to be more effective in reducing the number of CFUs, as the mean CFUs that remained after one-week application of TAP were 8 and 60.8 for aerobic and anaerobic CFUs, respectively. Other studies have also reported the efficacy of TAP in reducing total bacterial count and in treating primary teeth with pulpal exposure with poor prognosis (14, 28-31). Alfadda et al. investigated the antibacterial properties of triple TAP and CH

Table 1

Sample distribution, mean ± standard deviation, and percentage of reduction for aerobic and anaerobic micro-organisms before and after medicament application

	Before ICM Application (CFU/mL) mean± SD			After ICM Application (CFU/mL) mean± SD			Percentage of reduction mean± SD		
	TAP	NCH	Control	TAP	NCH	Control	TAP	NCH	Control
Aerobic bacteria	4.70 (±0.4) $\times 10^4$	4.07 (±0.49) $\times 10^4$	5.77 (±0.06) $\times 10^4$	8.00 (±0.18)	3.36 (±0.53) $\times 10^2$	4.48 (±0.38) $\times 10^4$	99.95 (±0.0)	98.05 (±2.36)	24.05 (±4.59)
p values	N.S*	N.S	N.S	0.00	0.00	0.00	0.00	0.00	0.00
Anaerobic bacteria	1.31 (±0.8) $\times 10^5$	1.29 (±0.52) $\times 10^5$	1.02 (±0.60) $\times 10^5$	6.08 (±0.73) $\times 10^1$	1.43 (±0.90) $\times 10^3$	0.64 (±0.70) $\times 10^5$	99.78 (±0.2)	90.79 (±1.69)	29.39 (±5.40)
p values	N.S	N.S	N.S	0.00	0.00	0.00	0.00	0.00	0.00

**N.S means Not Significant*

Table 2

The p values of the Mann-Whitney U test from the comparison of each two groups and the level of significance regarding the Mann-Whitney U test

Groups	Aerobic Before ICM application	Aerobic After ICM application	Anaerobic Before ICM application	Anaerobic After ICM application	Percent reduction of Aerobics	Percent reduction of Anaerobics
TAP and Control	0.72	0.00	0.45	0.00	0.00	0.00
NCH and Control	0.38	0.00	0.41	0.00	0.00	0.00
TAP and NCH	0.35	0.00	0.73	0.00	0.00	0.00

in root canals infected with *E. faecalis* biofilm. There was no significant difference between TAP and CH in the level of bacterial reduction, and they both improved viability and alkaline phosphatase activity in dental pulp stem cells on dentin surfaces (17). Dewi et al. determined the most effective concentrations of TAP and CH for complete eradication of *E. faecalis* within dentinal tubules. They reported that TAP at 10 mg mL^{-1} effectively eliminated bacteria, while CH was not successful in eradicating *E. faecalis* (20).

However, many in vitro and in vivo studies showed TAP few drawbacks such as human periodontal ligament fibroblasts cytotoxicity and notable inflammatory reactions in subcutaneous connective tissue. Furthermore, several studies showed overusing antibiotics such as TAP leads to the development of Antibiotic-resistant bacteria and antibiotic-resistant genes (32-34). Several studies have suggested that CH may not be effective against bacteria, particularly *E. faecalis*, but the nano form of CH improves its efficacy through greater penetration into the dentinal tubule depth compared to conventional CH. NCH showed a superior mean penetration depth at all coronal, middle, and apical dentin levels. Subsequently, nanoparticles' unique chemical and physical properties such as small dimensions and the highest surface area/mass ratio could be the fact behind the greater deep penetration, antibacterial activity, and long-lasting effects of NCH particles (35, 36). Recent studies also have supported this and showed that probable NCH particle agglomeration drawback was eliminated by combining with using high-intensity focused ultrasound (HIFU) to deliver in dentin tubules (37). Furthermore, an ex vivo study suggested the replacement of NCH as intracanal medicaments for the preservation of the root canal and dentin structure. They observed that CH could significantly reduce dentin microhardness in comparison with NCH, while NCH leads to any alteration in the microhardness value after 4 weeks (38).

Conclusion

Considering the total count of aerobic and anaerobic bacteria in primary tooth root canals, both TAP and NCH are more effective in reducing the number of CFUs during the endodontic treatment for a week. Moreover, no statistically significant difference was observed between the antibacterial activity of TAP and NCH. As regards, TAP use limitations such as antibiotic side effects and antimicrobial resistance, NCH could be a promising alternative to the treatment of primary teeth with pulpal infections. Further clinical trial studies with larger sample sizes are required to evaluate the long-term effects of this medicament on primary teeth and its efficacy in treating specific bacterial strains.

Clinical Relevance

Nano Calcium Hydroxide can be considered as an Intracanal Medicament in Human Primary Molars.

Declaration

Ethics approval

All procedures performed in this study followed the ethical standards of the institutional thesis research committee (Shiraz University of Medical Sciences IR.SUMS.REC.1397.828 and the randomized clinical trial was approved by the following code; (IRCT Code: IRCT20181226042132NI) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Conflict of Interest

The authors declare that they have no competing interests.

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