Jordan Journal of Dentistry, Volume **2**, No. **2**, **2025**
DOI: https://doi.org/10.14525/JJD.v2i2.06

Jordan Journal of Dentistry

www.jjd.just.edu.jo

Comparative Efficacy of Calcium Hydroxide Combined with Silver Nano-particles and Conventional Calcium Hydroxide as Intra-canal Medications in Reducing Post-operative Pain in Endodontic Retreatment: A Randomized Clinical Trial

Rawda Mohammad Abdel Rahman Baghdady [1], Raghda Mohammad Magdy [2], Reham Hammed Abu Anzah [3], Alaa Abd el Salam El Baz [4], Jealan Mohamed El-Shafei [4]

1 Faculty of Dentistry, Zagazig University, Zagazig, Egypt.
2 Faculty of Dentistry, Assuit University, Assuit, Egypt.
3 Private Clinic, Saudi Arabia.
4 Faculty of Dentistry, Cairo University, Cairo, Egypt.

ARTICLE INFO

Article History:
Received: 26/12/2024
Accepted: 15/2/2025

Correspondence:
Radwa M. Baghdady,
Faculty of Dentistry,
Zagazig University, Egypt.
rboghdady@dent.zu.edu.eg

ABSTRACT

Objectives: This research aims to evaluate the comparative efficacy of calcium hydroxide combined with silver nano-particles and conventional calcium hydroxide as intra-canal medications following non-surgical root canal retreatment in reducing post-operative pain.

Materials and Methods: A randomized, prospective, parallel, triple-blind clinical trial was conducted with 120 medically healthy patients diagnosed with symptomatic apical periodontitis. All patients had previously undergone endodontic treatment on anterior or premolar teeth and were between 25 years and 50 years old. Participants were randomly and equally assigned to two groups based on the added solution; either nano-silver solution or distilled water. Opaque bottles containing the nano-silver solution or distilled water were covered and coded as either A or B by a dental assistant, who prepared the intra-canal medicament paste by mixing one of the solutions with calcium hydroxide ($Ca(OH)_2$) powder before providing it to the operators. Both operators and patients were blinded to the group assignments. Patients were assessed pre-operatively and at 6, 12, 24, and 48 hours post-operatively using the Numerical Rating Scale (NRS). Statistical analyses were conducted using the Mann-Whitney U test, Wilcoxon's rank test, Friedman's test, and chi-square (χ^2) test. Additionally, relative risk (RR), relative-risk reduction (RRR), and 95% confidence intervals (CI) were calculated.

Results: There was no statistically significant difference in post-operative pain incidence and intensity between the two groups at different time intervals. However, within the same group, pain intensity varied significantly over time ($P<0.05$). The relative risk (RR) of pain incidence and its 95% CI at 6, 12, 24, and 48 hours were: 6 hours: RR = 0.86 (95% CI: 0.63, 1.19), 12 hours: RR = 0.87 (95% CI: 0.54, 1.41), 24 hours: RR = 0.54 (95% CI: 0.23, 1.26), 48 hours: RR = 0.33 (95% CI: 0.07, 1.59). No adverse effects related to the tested materials were reported.

Conclusions: Although the relative risk of pain incidence was lower in the treatment group than in the control group, the confidence intervals crossed 1, indicating no statistically significant difference. Further studies with larger sample sizes are needed to confirm these findings.

Keywords: Calcium hydroxide, Distilled water, Post-operative pain, Root-canal retreatment, Silver nano-particles.

1. Introduction

Pain may occur before, during, or after endodontic treatment and is a common concern for both endodontists and patients (1). Post-operative pain in root canal-treated teeth is defined as any degree of pain occurring after the initiation of root-canal treatment (2). The incidence of pain has been reported to range from 1.9% to 48% (3).

The causes of post-operative pain are complex and multi-factorial, often involving irritation or injury to peri-radicular tissues. A major contributor to severe pain after root-canal preparation is the extrusion of microbes and their by-products through the apex (4). Additionally, pain may occur depending on the extent of tissue damage and the nature of the damaging agent; whether microbial, mechanical, or chemical (5). However, the most common cause of pain is the presence of micro-organisms, such as *Enterococcus faecalis* (*E. faecalis*) and *Candida albicans* (*C. albicans*), which persist due to inadequate canal disinfection or reinfection resulting from an improper apical or coronal seal (6,7). These micro-organisms produce endotoxins and enzymes, inhibiting chemotaxis and phagocytosis, leading to the persistence of painful peri-apical lesions (1).

Therefore, anti-microbial agents used as intra-canal medications must be capable of penetrating dental tissues and achieving sufficiently high concentrations to eliminate the micro-organisms responsible for pulpal and peri-apical disease (8).

Calcium hydroxide has become one of the most commonly used intra-canal medicaments in managing multi-bacterial infections of root canals (9). It functions by altering bacterial-cell walls and denaturing potent endotoxins, such as lipopolysaccharides, making them less antigenic. Calcium hydroxide ($Ca(OH)_2$) has demonstrated anti-microbial properties, contributing to its pain-preventive effects (1). Additionally, it helps control the inflammatory process and promotes tissue repair (10). Its high pH (12.5-12.8) stimulates fibroblasts, neutralizes acidic environments, induces hard tissue formation, and acts as a physical barrier to prevent root-canal reinfection (11). Moreover, research has shown that calcium hydroxide significantly reduces the microbial population when applied in the root canal for at least one week (12,13).

Despite its unquestionable merits, $Ca(OH)_2$ suffers from various limitations, including poor anti-bacterial effects against specific pathogens, like *E. faecalis* and *Candida albicans*, dentin's ability to buffer the high pH environment affecting its anti-bacterial activity *in vitro*, limited volatility making it challenging to target micro-organisms in areas such as apical deltas and isthmuses, slow onset of action and removal difficulty (13).

Various vehicles have been added to calcium hydroxide to enhance its anti-bacterial activity, bio-compatibility, ionic dissociation into hydroxyl and calcium ions, and diffusion (14). The choice of vehicle, such as saline, camphorated mono-chlorophenol, antibiotics, chlorhexidine, glycerin, or propylene glycol, can either facilitate or inhibit the ionic dispersion from the paste (15).

Specific micro-organisms, such as *E. faecalis* and *C. albicans*, were found to be resistant to $Ca(OH)_2$ (16). Additionally, its inability to penetrate deeper layers of dentinal tubules is one of the drawbacks of $Ca(OH)_2$ (17). Hence, to enhance the effectiveness of $Ca(OH)_2$, substances with various anti-bacterial and chemical characteristics have been used as vehicles (11).

Nano-particles possess unique properties, such as greater charge density and a larger surface area of contact, compared to their bulk counterparts. As a result, significant interactions occur between their positively charged constituents and the negatively charged bacterial cell surfaces. The dimensions of nano-particles range from 1nm to 100 nm and can only be micro-scopically visualized (18).

Silver nano-particles (AgNPs), in particular, kill bacteria by damaging their cell walls. They create tiny holes in the membrane, allowing nano-particle accumulation on the cell surface. This increases membrane permeability, disrupts normal cellular function, and ultimately leads to bacterial cell death (19, 20).

Silver nano-particles exhibit anti-microbial activity, primarily due to their ability to generate free radicals. These radicals, originating from the nano-particle surface, cause bacterial cell membrane damage (19). Additionally, silver ions released from the nano-particles interact with the thiol groups of essential enzymes, leading to their inactivation (20). Moreover, as a soft acid, silver reacts with soft bases, such as the sulfur and phosphorus components of DNA, leading to DNA damage and bacterial cell death (20,21). Nano-particles can also modulate signal transduction, inhibiting bacterial growth (22). Consequently, they help alleviate pain which is often caused by microbial infections (23). Furthermore, their small size allows

deep penetration into dentinal tubules and areas of root canals that are typically inaccessible to conventional irrigation solutions (24). Accordingly, the anti-microbial efficacy of calcium hydroxide ($Ca(OH)_2$) is enhanced by the addition of silver nano-particles (25).

Several studies have assessed the anti-microbial effects of silver nano-particles (AgNPs) as an intra-canal medication, reporting favorable results (25-28). Regarding cytotoxicity, AgNPs have been found to be bio-compatible, particularly at lower concentrations (29). Therefore, this study aims to evaluate and compare the effects of calcium hydroxide ($Ca(OH)_2$) combined with AgNPs *versus* $Ca(OH)_2$ alone as intra-canal medications on post-operative pain following non-surgical endodontic retreatment of previously failed root canal-treated maxillary and mandibular anterior and premolar teeth with symptomatic apical periodontitis.

2. Materials and Methods

2.1 Sample Size

Sample-size calculation using GPower, based on 80% power and a Type-I (α) error probability of 0.05, determined that a total of 90 patients would be sufficient to detect a Cohen's effect size of 0.6. To account for a potential dropout rate of 30%, the total sample size was adjusted to 120 patients.

2.2 Randomization

Random sequence generation was performed using the permuted-block method, with three blocks of 40 participants each. A single operator treated all subjects within each block. Random sequence generation was conducted using Microsoft Excel. Within each block, participants were randomized and equally divided into two groups: 20 participants in Group A and 20 in Group B, maintaining an allocation ratio of 1:1.

To ensure blinding, a dental assistant, not involved in the research, prepared three boxes, each containing 40 folded papers. Near the end of the first visit, after chemo-mechanical preparation of the eligible teeth, patients were asked to draw a folded paper from the box. Based on the number drawn, the dental assistant assigned each patient to either Group A or Group B, according to the pre-generated random sequence.

Once assigned, the patient's name and ticket number were recorded in the randomization table. The patient's chart was coded by group to facilitate decoding during data management.

2.3 Trial Design

This study is a randomized, prospective, parallel, two-arm, superiority, triple-blind clinical trial with an allocation ratio of 1:1. It is also a phase-III therapeutic study conducted in accordance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines for randomized clinical trials in endodontics.

The Research Ethics Committee of the Faculty of Dentistry, Cairo University, approved the study protocol and informed consent process. The trial was registered at ClinicalTrials.gov. (ClinicalTrials.gov Identifier: NCT04213716).

2.4 Clinical Procedure

A total of 120 medically healthy patients, aged from 25 years to 50 years, with previously endodontically treated anterior or premolar teeth and symptomatic apical periodontitis-confirmed through clinical and radiographic diagnosis-were included in this study.

Pregnant females, patients requiring retreatment for more than one adjacent tooth (to exclude the possibility of pain referral), those who had taken oral medications within 72 hours before treatment, and individuals with badly decayed crowns were excluded.

After a detailed explanation of the procedures, benefits, and risks of the study, all included patients provided written informed consent. Patients were recruited from the outpatient clinics of the Endodontic Department, Faculty of Dentistry, Cairo University.

For all included patients, medical and dental histories were collected and recorded in pre-designed charts. The diagnostic criteria for endodontically treated teeth with symptomatic apical periodontitis were confirmed through clinical and radiographic examinations. Clinically, diagnosis was based on a painful response to palpation and/or percussion tests. Radiographically, peri-apical radiography was used for assessment, though peri-apical radiolucency may or may not have been present.

Treatment for all cases was completed in two sessions. In the first session, the patient was anesthetized using either a mandibular nerve block or infiltration technique, depending on the tooth's position in the upper or lower arch. The coronal restoration was removed under rubber dam isolation using a carbide fissure bur.

Gutta-percha was removed using a NiTi rotary retreatment system (ProTaper Retreatment Rotary System, Dentsply Sirona, Ballaigues, Switzerland),

following the manufacturer's recommended speed, torque, and sequence. The procedure began with D1, which penetrated the old gutta-percha in the coronal third of the canal, followed by D2 (with a non-cutting tip) for removal from the middle third, and finally D3 (also with a non-cutting tip) for removal from the apical third. A gutta-percha solvent (Carvene, Prevest DenPro, Ltd., Jammu, India) was used to facilitate the process.

After the removal of old gutta-percha, canal patency was established using manual K-files (#10 and #15) (Mani, Mani Inc., Tochigi, Japan). Working-length determination was performed using an electronic apex locator (Root ZX, J. Morita USA, Irvine, CA, USA).

Root canals were then prepared using NiTi rotary files (ProTaper Universal, Dentsply Sirona, Ballaigues, Switzerland) in an endodontic device (X-Smart, Dentsply Sirona, Ballaigues, Switzerland), following the manufacturer's recommendations. Root-canal preparation was completed using F3, F4, or F5 files, as needed.

The canal system was thoroughly irrigated with 3 mL of 2.5% sodium hypochlorite after each instrumentation step, with recapitulation using the initial file. Before each rotary instrument change, apical patency was confirmed using a #10 K-file. A 17% EDTA cream (MD-Chelcream, Meta Biomed Co., Ltd., Chungbuk, Korea) was used as a lubricant during instrumentation.

At the end of root-canal preparation, the canals were irrigated with 17% EDTA solution (Endo Solution, Cerkamed Medical Co., Poland) for 1 minute to remove the smear layer, followed by a final rinse with 5 mL of saline solution. The canal space was then dried using ProTaper Universal paper points (Dentsply Sirona, Ballaigues, Switzerland). Finally, the intra-canal medication paste was prepared according to the assigned experimental group.

2.4.1 Interventions

In Group A (silver nano-particles with calcium hydroxide powder), 0.1 g of calcium hydroxide powder (Prevest DenPro Ltd., Jammu, India) was mixed with 0.1 mL of a 30-ppm nano-silver solution containing spherical-shaped Ag^{+} nano-particles (average size: 20 ± 3 nm, concentration: 1 mM) (Nano Tech Egypt for Photo-Electronics, Al Giza, Egypt) in a 1:1 ratio.

In Group B, (conventional calcium hydroxide), 0.1 g of calcium hydroxide powder (Prevest DenPro Ltd., Jammu, India) was mixed with 0.1 mL of distilled water in a 1:1 ratio.

In both groups, the calcium hydroxide powder was mixed with either the nano-silver solution or distilled water on a glass slab using a spatula to form a paste. The medicaments were introduced into the canals using Lentulo spirals (Mani, Mani Inc., Tochigi, Japan), ensuring that the length was 2 mm shorter than the working length under aseptic conditions. Excess material was removed, and the access cavity was sealed with a temporary filling (MD-Temp, Meta Biomed Co., Ltd., Chungbuk, Korea).

Patients were instructed to contact the operators in case of severe pain between visits, in which case an emergency analgesic (Ibuprofen 400 mg, Novartis Pharmaceuticals, Egypt) would be prescribed.

In the second clinical session, after a 7-day interval, patients were anesthetized as in the first visit. Under rubber dam isolation, the temporary filling was removed, and the root canal was irrigated with 5 mL of sterile saline, followed by 1 mL of 20% citric acid to aid in the complete removal of the intra-canal dressing. A final rinse with 5 mL of sterile saline was performed to ensure complete removal of intra-canal dressing.

Before obturation, a clinical evaluation was conducted, including percussion, palpation, periodontal probing, as well as visual inspection for the absence of swelling, sinus tract, or fistula. A radiograph was taken to verify the position of the master cone at the predetermined working length.

The root canal was obturated using the cold lateral compaction technique (modified single-cone technique) with ProTaper Universal gutta-percha cones (Dentsply Sirona, Ballaigues, Switzerland) and AdSeal resin-based root-canal sealer (AdSeal, Meta Biomed Co., Ltd., Chungbuk, Korea). The appropriate master cone and auxiliary cones were used to ensure a proper seal.

A cotton pellet and a temporary filling (MD-Temp, Meta Biomed Co., Ltd., Korea) were placed, and the quality of the root-canal filling was assessed using a post-obturation peri-apical radiograph.

2.4.2 Blinding

Opaque bottles containing either the nano-silver solution or distilled water were covered and coded as A or B by the dental assistant, who prepared the intra-canal medicament paste according to the generated random sequence for allocation concealment. After chemo-

mechanical preparation and drying of the canal, the prepared paste was provided to the operators.

The mixed pastes had a similar color and were difficult to distinguish, as the silver nano-particle solution was light yellow. To maintain blinding, operators, participants, and outcome assessors were unaware of the group assignments.

2.5 Outcome Measures

Pre-operative pain was assessed using the Numerical Rating Scale (NRS), where patients recorded their pain levels on a scale from 0 to 10. (32) with 0 meaning "no pain" and 10 meaning "worst pain". The pain scores included four categories: 0 (none), 1-3 (mild), 4-6 (moderate) and 7-10 (severe).

A pain diary was provided to all patients to record their pain levels at 6, 12, 24, and 48 hours after the first visit of endodontic retreatment. Patients were instructed to mark their pain level on the Numerical Rating Scale (NRS) accordingly. Operators instructed all patients to contact them if they experienced severe pain between visits. In such cases, Ibuprofen 400 mg was prescribed for pain relief. At the second visit (one week later), the operator collected the pain diaries from the patients for assessment.

2.6 Statistical Analysis

Data was collected, tabulated, and coded for statistician blinding; by using Microsoft Excel sheets (Microsoft Corporation, Redmond, WA, USA). Descriptive analysis for all variables was carried out. Data was explored for normality using Kolmogorov–Smirnov test and Shapiro–Wilk test. Student's t-test was used for analysis of normally distributed, continuous variables, while the Mann–Whitney U-test was used for analysis of non-normally distributed continuous variables. Friedman's test was used for intra-group comparisons at various time periods, if statistical significance was detected. Wilcoxon's sign rank test was used for multiple comparisons. Pearson's chi-square ($\chi 2$) test was used for categorical variables. Relative Risk (RR) and its 95% confidence intervals (CI) calculated for risk of pain, for evaluation of risk of pain incidence, Relative Risk Reduction (RRR), Absolute Risk Reduction (ARR) and the number needed to treat NNT were estimated. A binary logistic regression (BLR) for the assessment of the relation between the incidence of post-endodontic pain (No/Yes) as a dependent variable and independent variables as gender (Female/Male), preoperative pain (No/Yes), age group (No/Yes), tooth type (No/Yes), tooth location (No/Yes), canal numbers (No/Yes) and analgesic intake (No/Yes) was carried out, and values were calculated for all variables and other factors remained constant. Values less than 0.05 for P-values were considered statistically significant. Data was analyzed using the Package for Social Sciences, version 25.0 for Windows (SPSS, IBM Corporation, NY, USA).

3. Results

Out of 158 enrolled participants, 120 patients met the inclusion criteria and were included in the study, with no dropouts or exclusions. Baseline characteristics of the 120 included patients are summarized in Table 1.

Baseline data was comparable between both groups ($P>0.05$), except for pre-operative pain incidence (Table 1). An intention-to-treat (ITT) analysis was used, ensuring that the participants remained in their originally assigned intervention group or control group.

Randomization and statistical analysis preserved prognostic balance, minimizing bias and providing an unbiased estimate of the intervention efficacy on the primary study outcome, based on the observed adherence levels in the trial.

After the application of intra-canal medicaments, pain intensity decreased at all time points in both groups, with no statistically significant difference between them ($P>0.05$). However, within each group, pain scores declined significantly over time ($P<0.001$) at different intervals, as shown in Table 2. The pain-intensity levels over time for both groups are clearly represented in Figure 1.

The pain-incidence levels at the different pain categories and at the different time intervals are presented in Table 3. At 6 hours post-operatively, the relative risk (RR) of pain incidence, regardless of its intensity, was 0.861 (95% confidence interval [CI]: 0.625, 1.186), Also, Relative Risk Reduction (RRR) of pain incidence, regardless of its intensity, was 14% on using AgNPs combined with calcium hydroxide compared to calcium hydroxide alone, while, Absolute Risk Reduction (ARR) was 8.3%. The Number Needed to Treat (NNT) was 12; (meaning that 12 patients need to receive AgNPs instead of conventional calcium hydroxide for one additional patient to avoid post-operative pain). At 12 hours post-operatively, RR was

0.87 (95% CI: 0.53, 1.41), RRR of pain incidence was 13%, and ARR was 5%. The NNT was twenty. At 24 hours post-operatively, RR was 0.53 (95% CI: 0.23, 1.25), RRR of pain incidence was 46%, ARR was 10%. The NNT was 10. At 48 hours post-operatively, RR was 0.33 (95% CI: 0.07, 1.58), Relative Risk Reduction of pain incidence was 67% and ARR was 7%. The NNT was 15.

Table 1: Baseline characteristics of the included study participants for AgNPs- calcium hydroxide combination and $Ca(OH)_2$ groups

Variable	AgNPs (n,%)	$Ca(OH)_2$ (n, %)	P-value
Age (years) (n=60)			
Mean ± SD	35.3±8.0	36.7±8.0	0.34
Range	25-50	25-50	
Gender			
Female	47 (78.3)	47 (78.3)	1.00
Male	13 (21.7)	13 (21.7)	
Tooth type			
Anterior	25 (41.7)	23 (38.3)	0.71
Premolar	35 (58.3)	37 (61.7)	
Tooth location			
Mandibular	14 (23.3)	12 (20)	0.66
Maxillary	46 (76.7)	48 (80)	
Number of canals			
One	38 (63.3)	43 (71.7)	0.33
Two	22 (36.7)	17 (28.3)	
Pre-operative pain incidence			
Moderate	18 (30)	31(51.7)	0.02
Severe	42 (70)	29 (48.3)	
Overfilling post-operative error			
Yes	3 (5)	4 (6.7)	1.00
No	57 (95)	56 (93.3)	

SD: Standard Deviation.

Table 2: Pain intensity for AgNPs- calcium hydroxide combination and $Ca(OH)_2$ groups

	AgNPs (n = 60)					**Ca $(OH)_2$ (n = 60)**					P-value 1[a]
Time	Med	IQR	Min.	Max.	Mean (SD)	Med	IQR	Min.	Max.	Mean (SD)	
PreOP	7[a]	2	4	10	7 (1)	7[a]	2	5	10	7 (2)	0.21
POP6 h	1[A]	3	0	10	2 (2)	2[A]	4	0	10	2 (3)	0.34
POP12 h	0[b-B]	2	0	7	1 (2)	0[b-B]	2	0	6	1 (2)	0.53
POP24 h	0[b-B]	0	0	4	0 (1)	0 [b-B]	0	0	5	1 (1)	0.16
POP48 h	0[b-B]	0	0	2	0 (0)	0 [b-B]	0	0	2	0 (0)	0.16
P-value 2[b]			<0.001					<0.001			

[a] Mann–Whitney test comparing two independent groups in a row.

[b] Friedmann's test comparing different time points within each group in a column; different lower-case letters within each column represent a statistically significant difference of each post-operative time-point from the pre-operative time point within each group (Wilcoxon's sign rank test); different upper-case letters within each column represent a significant difference of each time point from the one preceding it within each group (Wilcoxon's sign rank test).

[c] Statistical significance ($P < 0.05$).

Med, median; IQR, interquartile range; Min., minimum; Max., maximum; SD, standard deviation. PreOP, pre-operative pain; POP6 h, pain after 6 hours; POP12 h., pain after 12 hours; POP24h, pain after 24 hours; POP48h., pain after 48 hours.

Since the relative risk (RR) of pain incidence at all post-operative time points (6, 12, 24, and 48 hours) was less than 1, with 95% confidence intervals crossing 1, the clinical significance of AgNPs mixed with calcium

hydroxide as an intra-canal medication is evident. This combination was found to reduce post-operative pain at different follow-up periods (6, 12, 24, and 48 hours) more effectively than calcium hydroxide alone. However, to establish a statistically significant difference between the two groups, further studies with a larger sample size are recommended.

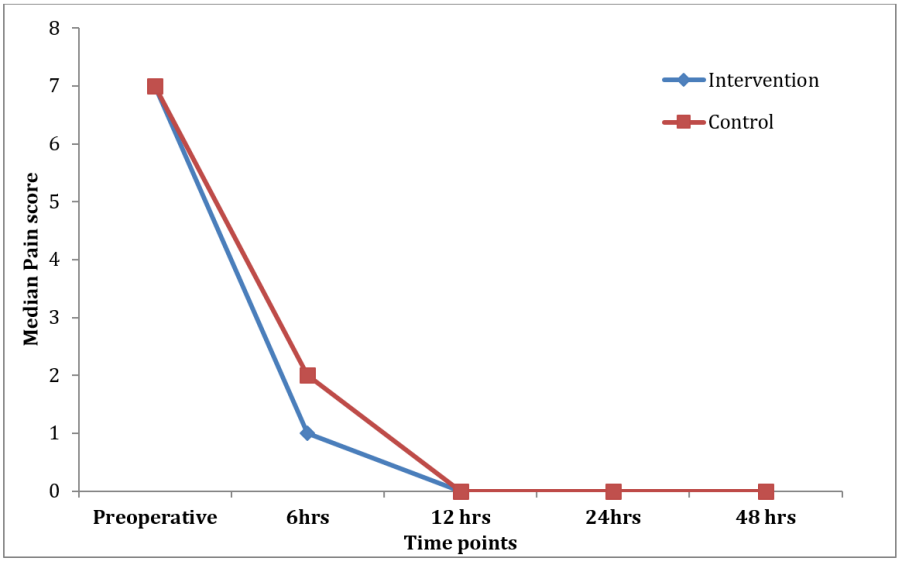

Figure 1: Intensity level of pain for AgNPs- calcium hydroxide combination and $Ca(OH)_2$ groups

Table 3: Incidence [n (%)] of pain in the different pain categories for AgNPs- calcium hydroxide combination and $Ca(OH)_2$ group

Time	Pain category	AgNPs (n,%)	$Ca(OH)_2$ (n,%)	P- value
PreOP	Moderate	18 (30)	31 (51.7)	0.02 *
	Severe	42 (70)	29 (48.3)	
POP6 h	No Pain	29 (48.3)	24 (40)	0.10
	Mild	22 (36.7)	20 (33.3)	
	Moderate	3 (5)	12 (20)	
	Severe	6 (10)	4 (6.7)	
POP12 h	No Pain	40 (66.7)	37 (61.7)	0.67
	Mild	13 (21.7)	15 (25)	
	Moderate	6 (10)	8 (13.3)	
	Severe	1 (1.7)	0	
POP24 h	No Pain	53 (88.3)	47 (78.3)	0.17
	Mild	5 (8.3)	12 (20)	
	Moderate	2 (3.3)	1 (1.7)	
POP48 h	No Pain	58 (96.7)	54 (90)	0.27
	Mild	2 (3.3)	6(10)	

* Statistically significant.
PreOP, pre-operative pain; POP6h, pain after 6 h; POP12h, pain after 12 h; POP24h, pain after 24h; POP48h, pain after 48 h.

The Binary Logistic Regression analysis indicated that the overall post-operative pain incidence (No/Yes), when analyzed in relation to independent variables such as gender, age, tooth type, tooth location, canal number, and pre-operative pain, showed no statistically significant difference (P >0.05) (Table 4).

Table 4: Binary linear regression of the effect of different variables on overall post-operative pain

Variables		Overall POP				
		No Pain (N=46)		Pain(N=74)		P-value
		Count	%	Count	%	
Groups	AgNPs	26	43.3	34	56.7	0.26
	$Ca(OH)_2$	20	33.3	40	66.7	
Age Group	≤35	23	39	36	61	0.88
	>35	23	37.7	38	62.3	
Gender	Females	33	35.1	61	64.9	0.16
	Males	13	50	13	50	
Tooth type	Anterior	21	43.8	27	56.3	0.31
	Premolar	25	34.7	47	65.3	
Tooth location	Mandibular	13	50	13	50	0.16
	Maxillary	33	35.1	61	64.9	
Canal numbers	One	35	43.2	46	56.8	0.11
	Two	11	28.2	28	71.8	
Pre-operative pain	Moderate	23	46.9	26	53.1	0.10
	Severe	23	32.4	48	67.6	

$P < 0.05$: statistically significant; POP: post-operative pain.

4. Discussion

Post-operative pain includes root-canal treatment flare-ups which represent severe pain with or without swelling occurring after the initiation or continuation of endodontic treatment (33). Its most common cause is micro-organisms, such as *E. faecalis* and *C. albicans* (1, 23). Chemo-mechanical preparation aims to reduce or eliminate microbial infection from the root canal system. Chemical preparation involves irrigation and intra-canal dressings, with calcium hydroxide being one of the most commonly used intra-canal medications (34).

Calcium hydroxide exerts its action through ionic dissociation into Ca^{2+} and OH^- ions when combined with an aqueous vehicle. These ions contribute to hard-tissue deposition and exhibit a potent anti-microbial effect. (35). The anti-microbial activity of calcium hydroxide has been shown to improve when combined with chlorhexidine or silver nano-particles, leading to a reduction in post-operative pain (1, 25, 26, 36).

Silver nano-particles exhibit excellent anti-microbial activity and possess anti-inflammatory properties. They reduce local matrix metalloproteinase activity, promote neutrophil apoptosis, and lower levels of pro-inflammatory cytokines by inhibiting the actions of interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α); both being key players in inflammation. These anti-inflammatory effects make nano-silver a promising candidate for use as an anti-inflammatory agent in various therapeutic applications (20).

Analysis of patients' gender, age, tooth type, tooth location, and number of canals showed a similar distribution between the two groups, confirming proper randomization. The contribution of these factors to post-operative pain incidence was not significant. This finding is in harmony with Imura et al. (1995) (37) and in contrast with Nagendrababu and Gutmann (2017) (38). The use of strict eligibility criteria, a standardized protocol, and patient randomization minimized the impact of both known and unknown variables on the study results (39).

The present study demonstrated that the calcium hydroxide combined with silver nano-particle solution group experienced a significant reduction in post-operative median pain scores across all time periods compared to pre-operative levels. Additionally, the pain intensity declined progressively over time, with statistically significant differences observed between time intervals (6, 12, 24, and 48 hours). These results are in agreement with those of previous studies (25, 26, 36, 40), but they disagree with those of others (27, 41). The differences may be attributed to the nano-sized structure

of the AgNPs combined with calcium hydroxide used in this study. The significant reduction in pain intensity could be explained by its potent anti-bacterial activity against potential pathogenic micro-organisms (42) and its ability to deeply penetrate dentine tubules, enhancing anti-microbial effectiveness (43). Since *E. faecalis* is commonly found in high counts in secondary apical lesions and can deeply penetrate dentinal tubules, it may evade the effects of conventional intra-canal procedures. Additionally, *E. faecalis* exhibits resistance to the high pH of calcium hydroxide ($Ca(OH)_2$), making it more resistant to $Ca(OH)_2$ alone (44). Siqueira et al. (2002) reported that the most common cause of post-operative pain is micro-organisms (23) and Vineet et al. (2016) stated that there is a relationship between endodontic signs and symptoms and tenderness on percussion with specific endodontic pathogen (*E. faecalis)* (45).

In the present study, both groups produced comparative relief of endodontic pain; thus, the differences were not statistically significant regarding pain intensity and pain incidence at different follow-up periods. However, despite the lack of statistical differences, at 6 hours post-operatively, calcium hydroxide combined with AgNps and calcium hydroxide groups achieved 85% and 73% pain relief, respectively. It is needless to say that the clinical significance may not be strictly aligned with statistical significance. Thus, any further degree of pain alleviation may be considered clinically significant to the patients. The results of this study corroborated the findings documented by Javidi et al. (2014), and Pourhashemi et al. (2015) (25, 26). These findings may be explained by the fact that $Ca(OH)_2$ combined with AgNPs solution has improved and amplified the $Ca(OH)_2$ anti-microbial effect on both planktonic and intra-canal biofilms of intra-canal micro-organisms (18, 28, 40).

A limitation of this trial is that the outcome was subjective. Future trials with the same aim are recommended. Also, it is recommended to measure and correlate anti-microbial activity (objective outcome) with post-operative pain (subjective outcome) on a wider scale of patients than that used in this trial.

5. Conclusions

Using calcium hydroxide silver nano-particulate combinations is effective as intra-canal medicaments in symptomatic apical periodontitis with previously endodontically treated cases, as they decrease post-operative pain incidence by time, especially after 6 hours as compared to conventional calcium hydroxide. So, within the limitations of the current study, this combination (calcium hydroxide with nano-silver solution) can be recommended for use with endodontic retreatment cases.

Acknowledgements

The authors would like to extend their gratitude to the Endodontic Department, Faculty of Dentistry, Cairo University for the valuable assistance in completing the current research.

Conflict of Interests

The authors have no conflict of interests to declare.

Funding Information

This research did not receive any funding from any source.

References

1. Singh RD, Khatter R, Bal RK, Bal CS. Intra-canal medications *versus* placebo in reducing post-operative endodontic pain: A double-blind randomized clinical trial. Braz Dent J. 2013;24:25-29.
2. Menakaya IN, Oderinu OH, Adegbulugbe IC, Shaba OP. Incidence of post-operative pain after use of calcium hydroxide mixed with normal saline or 0.2% chlorhexidine digluconate as intra-canal medicament in the treatment of apical periodontitis. Saudi Dent J. 2015;27:187-193.
3. Ali SG, Mulay S, Palekar A, Sejpal D, Joshi A, et al. Prevalence of and factors affecting post-obturation pain following single visit root canal treatment in an Indian population: A prospective, randomized clinical trial. Contemp Clin Dent. 2012;3:459-463.
4. Siqueira Jr JF. Microbial causes of endodontic flare-ups. Int Endod J. 2003;36:453-463.
5. Anjaneyulu K, Nivedhitha MS. Influence of calcium hydroxide on the post-treatment pain in endodontics: A systematic review. J Conserv Dent. 2014;17:200-207.
6. Abouelenien SS, Ibrahim SM, Shaker OG, Ahmed GM. Evaluation of post-operative pain in infected root canals after using double antibiotic paste *versus* calcium hydroxide as intra-canal medication: A

randomized controlled trial. F1000Res. 2018;7:1-9.
7. Sritharan A. Discussing that the coronal seal is more important than the apical seal for endodontic success. Aust Endod J. 2002;28:112-115.
8. Athanassiadis B, Abbott P V, Walsh LJ. The use of calcium hydroxide, anti-biotics and biocides as anti-microbial medicaments in endodontics. Aust Dent J. 2007;52:S64-S82.
9. Gangwar A. Anti-microbial effectiveness of different preparations of calcium hydroxide. Indian J Dent Res. 2011;22:66-70.
10. Silva LAB, Nelson-Filho P, Leonardo MR, Rossi MA, Pansani CA. Effect of calcium hydroxide on bacterial endotoxin *in vivo*. J Endod. 2002;28:94-98.
11. Silveira CFDM, Cunha RS, Fontana CE, de Martin AS, Gomes BPFDA, et al. Assessment of the anti-bacterial activity of calcium hydroxide combined with chlorhexidine paste and other intra-canal medications against bacterial pathogens. Eur J Dent. 2011;5:1-7.
12. Shuping GB, Orstavik D, Sigurdsson A, Trope M. Reduction of intra-canal bacteria using nickel-titanium rotary instrumentation and various medications. J Endod. 2000;26:751-755.
13. Zou X, Zheng X, Liang Y, Zhang C, Fan B, et al. Expert consensus on irrigation and intra-canal medication in root canal therapy. Inter J Oral Sci. 2024;16:23.
14. Pacios MG, Silva C, López ME, Cecilia M. Anti-bacterial action of calcium hydroxide vehicles and calcium hydroxide pastes. J Investig Clin Dent. 2012;3:264-270.
15. Vianna ME, Paula B, Almeida F De, José F, Filho DS. *In vitro* evaluation of the susceptibility of endodontic pathogens to calcium hydroxide combined with different vehicles. Braz Dent J. 2005;16:175-180.
16. Delgado RJR, Gasparoto TH, Sipert CR, Pinheiro CR, de Moraes IG, et al. Anti-microbial activity of calcium hydroxide and chlorhexidine on intra-tubular *Candida albicans*. Int J Oral Sci. 2013;5:32-36.
17. Adl A, Hamedi S, Sedigh Shams M, Motamedifar M, Sobhnamayan F. The ability of triple anti-biotic paste and calcium hydroxide in disinfection of dentinal tubules. Iran Endod J. 2014;9:123-126.
18. Fahim MM, Saber SEM, Elkhatib WF, Nagy MM, Schafer E. The anti-bacterial effect and the incidence of post-operative pain after the application of nano-based intra-canal medications during endodontic retreatment: A randomized controlled clinical trial. Clin Oral Investig. 2022;26:2155-2163.
19. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, et al. Anti-microbial effects of silver nano-particles. Nanomed. 2007;3:95-101.
20. Prabhu S, Poulose EK. Silver nano-particles: Mechanism of anti-microbial action, synthesis, medical applications, and toxicity effects. Int Nano Lett. 2012;2:1-10.
21. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, et al. The bactericidal effect of silver nano-particles. Nano-technol. 2005;16:2346-2353.
22. Shrivastava S, Bera T, Roy A, Singh G, Ramachandrarao P, et al. Characterization of enhanced anti-bacterial effects of novel silver nano-particles. Nano-technol. 2007;18:1-9.
23. Siqueira JF, Rocas IN, Favieri A, Machado AG, Gahyva SM, et al. Incidence of post-operative pain after intra-canal procedures based on an anti-microbial strategy. J Endod. 2002;28:457-460.
24. ElKateb WM, Massoud AG, Mokhless NA, Shalaby TI. Measurement of tubular penetration depth of three types of nano-particles mixed with endodontic sealer using scanning electron microscope: An *in vitro* study J Am Sci. 2015;11:111-122.
25. Javidi M, Afkhami F, Zarei M, Ghazvini K, Rajabi O. Efficacy of a combined nano-particulate/calcium hydroxide root canal medication in the elimination of *Enterococcus faecalis*. Aust Endod J. 2014;40:61-65.
26. Pourhashemi SJ, Afkhami F, Sadegh M, Salehi Y, Fard MJK. Anti-biofilm efficacy of silver nano-particles as a vehicle for calcium hydroxide medicament against *Enterococcus faecalis*. J Dent. 2015;43:1573-1579.
27. Alabdulmohsen Z, Saad A. Anti-bacterial effect of silver nano-particles against *Enterococcus faecalis*. Saudi Endod J. 2017;7:29-35.
28. Abdou SA, Kataia MA, Ali MMM, Hassan RS, Negm SI. Assessment of anti-microbial efficacy of silver nano-particles *versus* calcium hydroxide as intra canal medication: *In vivo* Study. Int J Microbiol Res. 2017;8:37-42.
29. Gomes-Filho JE, Silva FO, Watanabe S, Angelo Cintra LT, Tendoro KV, et al. Tissue reaction to silver nano-particle dispersion as an alternative irrigating solution. J Endod. 2010;36:1698-1702.
30. Cohen J. Statistical power analysis. Curr Dir Psychol Sci. 1992;1:98-101.
31. Rodig T, Vogel S, Zapf A, Hulsmann M. Efficacy of different irrigants in the removal of calcium hydroxide from root canals. Int Endod J. 2010;43:519-527.

32. Hjermstad MJ, Fayers PM, Haugen DF, Caraceni A, Hanks GW, et al. Studies comparing numerical rating scales, verbal rating scales, and visual analogue scales for the assessment of pain intensity in adults: A systematic literature review. J Pain Symptom Manag. 2011;41:1073-1093.
33. Tsesis I, Faivishevsky V, Fuss Z, Zukerman O. Flare-ups after endodontic treatment: A meta-analysis of literature. J Endod. 2008;34:1177-1181.
34. Siqueira Jr, Guimaraes-Pinto T, Rocas I.N. Effects of chemo-mechanical preparation with 2.5% sodium hypochlorite and intra-canal medication with calcium hydroxide on cultivable bacteria in infected root canals. J Endod. 2007;33:800-805.
35. Kim D, Kim E. Anti-microbial effect of calcium hydroxide as an intra-canal medicament in root canal treatment: A literature review - Part I. *In vitro* studies. Restor Dent Endod. 2014;39:241-252.
36. Zhang F hua, Li M, Wei Z jun, Zhao B. The effect of a combined nano-particulate/calcium hydroxide medication on the bio-film of *Enterococcus faecalis* in the starvation phase. Shanghai Kou Qiang Yi Xue. 2016;25:11-15.
37. Imura N, Zuolo ML. Factors associated with endodontic flare-ups: A prospective study. Int Endod J. 1995;28:261-265.
38. Nagendrababu V, Gutmann JL Factors associated with post-obturation pain following single-visit non-surgical root canal treatment: A systematic review. Quintessence Int. 2017;48:193-208.
39. Moher D, Hopewell S, Schulz KF, Montori V, Gøtzsche PC, et al. CONSORT 2010 explanation and elaboration: Updated guidelines for reporting parallel-group randomized trials. BMJ. 2010;340:1-28.
40. Hegazi MS, Ali MM, Hassan REL. Evaluation of anti-microbial effect of conventional calcium hydroxide, calcium-hydroxide nano-particles and combined calcium hydroxide with silver nano-particles as intra-canal medications against *Enterococcus faecalis*. Indian J Public Health Res Dev. 2019;10:2107-2112.
41. Mozayeni MA, Haeri A, Dianat O, Jafari AR. Anti-microbial effects of four intra-canal medicaments on Enterococcus Faecalis: An in vitro study. Iran Endod J. 2014;9:195-198.
42. Krishnan R, Arumugam V, Vasaviah SK. The MIC and MBC of silver nano-particles against *Enterococcus faecalis*: A facultative anaerobe. J Nanomed Nanotechnol. 2015;6:1-4.
43. Mohammadi Z, Soltani MK, Shalav S. An update on the management of endodontic bio-films using root canal irrigants and medicaments. Iran Endod J. 2014;9:89-97.
44. Pirani C, Bertacci A, Cavrini F, Foschi F, Acquaviva GL, et al. Recovery of *Enterococcus faecalis* in root canal lumen of patients with primary and secondary endodontic lesions. New Microbiol. 2008;31:235-240.
45. Vineet RV, Nayak M, Kotigadde S. Association of endodontic signs and symptoms with root canal pathogens: A clinical comparative study. Saudi Endod J. 2016;3:8-12.