RESEARCH PAPER

Effect of Bromelain as An Intracanal Medication on the Intensity of Postoperative Pain and Bacterial Reduction: A Randomized Clinical Trial

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Abstract

Objective This study evaluated the effect of bromelain versus calcium hydroxide as an intracanal medicament on the severity of postoperative pain, bacterial reduction, enterococcal count, and incidence of interappointment swelling.

Material and methods Thirty patients with single-rooted lower premolars with necrotic pulps and symptomatic apical periodontitis without periapical radiolucency were randomly divided into two groups based on the intracanal medication used: Bromelain group and Calcium hydroxide group. Under aseptic conditions, the pretreatment sample was taken using a sterile paper point dipped in brain heart infusion broth after access opening and working length determination. Post-instrumentation sample was taken after chemo-mechanical preparation. Intracanal medicament was placed for one-week, post-instrumentation pain was recorded at 6-, 12-, 24-, and 48-hours using the Numeric Rating Scale and interappointment swelling was recorded in the form of binary data (yes or no) up to 48-hours postoperatively. After 7 days, the post-medicament sample was collected. All samples were incubated aerobically for 48-h and plated on blood agar for total aerobic colony count and bile esculin agar for enterococcal colony, and CFU/ml was counted.

Results There was no statistically significant difference between both groups concerning the incidence and intensity of postoperative pain at all follow-up periods, intracanal bacterial reduction, Enterococcal colony count, and interappointment flare-up ($p > 0.05$).

Conclusions Within the limitations of this study, the findings provide preliminary evidence supporting the potential of bromelain as an intracanal medicament for reducing intracanal bacterial load, severity of postoperative pain, and incidence of interappointment swelling. However, further clinical trials with larger sample size are required to confirm its clinical effectiveness and safety relative to calcium hydroxide.

Keywords: Bromelain; Calcium hydroxide, Intracanal medication, Postoperative pain, Bacterial load reduction, Necrotic premolars.

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Introduction

Most of endodontic patients experience unpleasant postoperative pain following root canal treatment which varies between 3 - 58% that could lower the patient's life quality [1]. One of the contributing factors for this pain is the microbial etiology of peri radicular diseases [2]. Therefore, root canal treatment should be based on antibacterial strategies and disinfection protocols to lower the incidence of this pain [3].

In the literature it was evident that significant parts of the root canal walls remain untouched after mechanical instrumentation such the isthmuses, lateral canals, and infected dentinal tubules. Consequently, it appears that chemical irrigations and intracanal medications are required to eradicate contaminated tissues and bacteria [4]. Root canal failure is assumed to be due to the persistence of bacteria in the apical region of the root canals. Enterococcus faecalis (E. faecalis) has been found in 38% of the failed root canal-treated teeth which can be attributed to high alkalinity resilience and tubular attack capacity of these bacteria which protect it from intracanal endodontic medicaments [5].

Calcium hydroxide is the gold standard of intracanal medicaments in endodontic therapy. Studies have shown that its antibacterial efficacy is attributed to its high alkalinity due to the release of active hydroxyl ions in presence of liquid. These ions cause DNA damage, protein denaturation, and bacterial cytoplasmic membrane damage [6]. However, it has been shown to weaken the root canal system with limited capacity to eradicate certain species from the root canal system, making it less effective against the most resistant Enterococcus faecalis and Candida species [7,8]. Moreover, Calcium hydroxide is usually difficult to be removed even after extensive irrigation, leaving a residue that covers 20% to 45% of the canal wall leading to poor sealing ability and microleakage after obturation [9]. As a result, endodontics is now conducting extensive research to find a substitute for calcium hydroxide.

Many herbal agents have been used to disinfect the canals due to their strong antimicrobial efficacy, safety, and cost-effectiveness [10]. Bromelain is a proteolytic enzyme belonging to the Bromeliaceae family and obtained from the fruit and stem part of Ananas comosus, pineapples [11]. It possesses antithrombotic, antiedematous, anti-inflammatory, fibrinolytic, and antibacterial activities. The chemical components of bromelain, particularly flavonoids thought to be responsible for its antibacterial property, use hydrogen bonding to create strong interactions with extracellular proteins, changing the permeability of cell membranes [12].

To date, only a small number of studies in endodontics have used the naturally occurring bromelain pineapple extract with its proteolytic, anti-inflammatory, and antibacterial properties as an intracanal medicament [13-15]. Thus, the purpose of this study was to evaluate the effect of bromelain versus calcium hydroxide as an intracanal medicament on the severity of postoperative pain, bacterial load reduction, enterococcal count, and incidence of interappointment swelling in single-rooted lower premolar with necrotic pulps.

Materials and method

Study design, Registration, Setting

This randomized clinical trial has been written according to CONsolidated Standards Of Reporting Trials (CONSORT) 2025 guidelines [16]. The study was designed as a parallel, randomized, prospective controlled clinical trial with a superiority framework. The protocol for the study has been registered at (www.clinicaltrials.gov) (ID: NCT05687760) on (18/1/2023) after its approval by the Faculty of Dentistry's institutional review board and ethical committee (approval number 1223). Patients were recruited and treated in the endodontic clinic at the Faculty of Dentistry, Cairo University in the time between August 2023 to November 2024. All patients were fully informed about the study's purpose, treatment procedures, potential side effects, and available alternatives to treatment before signing an informed consent form.

Sample size

Using a methodology described in a previous study [17], the standard deviation of postoperative pain of Ca $(OH)_2$ was 0.79 at 48 hours, assuming a minimal clinically significant difference of 1. 22 patients (11 per group) were required with 5 % significance level and 80% power in order to reject the null hypothesis that the population proportions are equal. To compensate for the possible loss 35 % was added, so the sample size was raised to 30 patients (15 in each group). Sample size was estimated using Power and Sample Size Calculation software version 3.1.6.

Randomization and allocation concealment

A random numerical sequence (1–30) was made by the co-supervisor (MM) using computer software (http://www.random.org/). Two columns (A & B) with a 1:1 allocation ratio was designated for the calcium hydroxide (CH) group and the bromelain (BR) group, respectively. The table of randomization was maintained and only accessed by the co-supervisor (MM). Following mechanical preparation, patients were given opaque, closed envelopes containing eight-folded, numbered papers indicating the intracanal medication placed. After contacting (MM), the operator opened the envelope and administered the intracanal medication that was designated for that patient based on the number inside.

Blinding

Double blinding was accomplished in this trial by blinding the patients to the intervention group and the outcome assessor. However, blinding the operator was not feasible due to the nature of the intervention. To prevent any further bias, the operator was not aware of the treatment group until mechanical preparation is completed.

Eligibility criteria

The inclusion criteria were male and female patients aged between 20 to 60 years with mandibular single-rooted canal premolars diagnosed with pulp necrosis and symptomatic apical periodontitis showed radiographically slight widening in the periodontal membrane space without distinct periapical radiolucency. Also, patients without any notable medical history were considered for inclusion.

Conversely, patients with known systemic conditions, pregnant women, patients who took antibiotics within the 12 hours preoperatively, and patients who reported bruxism or clenching were excluded from participation in the study; since they had a lower healing rate and a higher pain perception [18].

Additionally, teeth that required additional procedural steps or a multidisciplinary approach were excluded as individuals with open apex, radiographic evidence of internal or external root resorption, vertical root fracture, perforation, or calcification; Miller's mobility index Grade II or III; swelling or sinus tract; acute periapical abscess; pocket depth greater than 5 mm; or previous root canal treatment.

Diagnosis

Mandibular single-rooted premolar with necrotic pulp was diagnosed based on the patient's history of no pain response to hot or cold stimuli, as well as a negative response to both the electrical pulp test (DY310 Dental Pulp Tester; Denjoy, Henan, China) and cold pulp test utilizing Endo ice (Coltene /Whale dent Inc., Cuyahoga Falls, OH, USA). The prior diagnostic tests were validated using the contralateral tooth as a control. Radiographic examination utilizing the Digora intraoral periapical sensor plate and software (Digora; Soredex, Helsinki, Finland) showed radiographically slight widening in the periodontal membrane space without distinct periapical radiolucency in mandibular canaled premolars.

Endodontic procedure

All patients received endodontic treatment over two visits, during which the tooth was anesthetized using the mental nerve block approach utilizing 1.8 milliliters of 2% Mepivacaine HCl with 1:100,000 epinephrine (Mepecaine-L, Alexandria Company for Pharmaceuticals and Chemical Industries, Egypt). All caries and/or coronal restorations were completely removed with a sterile bur after applying rubber dam. The operative field was thoroughly cleaned, including the tooth, the clamp, and rubber dam sheet, with 30% hydrogen peroxide (LUNA Perfumes and Cosmetics, 6th of October, Giza, Egypt) until no further bubbling of the peroxide occurred. All surfaces were then disinfected by a sterile cotton swab with a 5.25% sodium hypochlorite solution (NaOCl) (Clorox; 10th of Ramadan, Cairo, Egypt). The access cavity was then prepared using another sterile round carbide bur size 3 (Dentsply Sirona, Ballaigues, Switzerland**)** and Endo-Z bur (Dentsply Sirona). The operation field and pulp chamber were disinfected again using the previously mentioned technique.

To facilitate the insertion of paper points into the canal, the canal orifices were enlarged using hand SX files (Dentsply Sirona). The pre-instrumentation root canal samples (S1) were obtained by successively inserting three sterile paper points into the canal for one minute to absorb the fluid to a level that was roughly 1 mm short of the radiographic apex, transferred to a sterile bottle containing brain heart infusion broth.

Stainless steel hand K-files in sizes #10 and #15 (MANI, INC., Tochigi, Japan) were used to scout and negotiate the canals. Working length (WL) was determined using an electronic apex locator (Root ZX; J. Morita USA, Irvine, CA) and confirmed radiographically. Mechanical preparation was carried using nickel–titanium rotary M-Pro files with X smart plus motor (Dentsply Maillefer) starting by using orifice opener file (#18/.09), 3 mm shorter than the WL, followed by #20/.04, #25/.06 up to full WL and finishing with file #35/.04. Canals were irrigated with 2.5% sodium hypochlorite solution (5 ml) between each two successive instruments using a disposable plastic syringe with side-ventilated 30-gauge needle (Steri irrigation tips; Diadent, Seoul, Korea).

As previously mentioned, post-instrumentation root canal samples (S2) were obtained from the canals, and within 15 minutes, the microbiological samples were sent to the microbiology lab. Based on the intracanal medication placed, root canals were randomly assigned into two groups, each of which contained 15 samples:

In the BR group, Bromelain paste was prepared by mixing bromelain powder (Acros organics BV., Geel., Belgium) derived from the stem with an enzyme activity of 2400 gelatin digestion units per gram with saline in a 1:1 proportion, meaning that 1 gram of powder was mixed with 1 ml of distilled saline [13-15]. The resultant paste was placed in the canal using a sterile plastic syringe with a 27-gauge needle without binding 1 mm shorter than the WL. The access cavities were sealed using sterile cotton pellet and glass ionomer cement (Ketac Molar; 3M ESPE, Bavaria, Germany).

In the CH group, calcium hydroxide (Meta Biomed, Cheongju, Korea) was injected inside the canals 1 mm shorter than working length. The access cavities were sealed using the same protocol.

The second visit was scheduled after one week, isolation was done, and the tooth was once again disinfected intracanal medication was removed by copious irrigation of 10 ml saline followed by taking the third sample (S3) in the same way as before. Master cone of a 0.40 taper gutta-percha (Meta Biomed) was fitted and verified radiographically to WL.

Root canal was irrigated with 5 mL of 2.5% sodium hypochlorite (NaOCl) for 1 minute, followed by 5 mL of 17% ethylenediaminetetraacetic acid (EDTA) for 1 minute. Subsequently, ultrasonic activation was performed using Ultra X (Eighteeth, Changzhou, China) for 10 seconds. The canal was then rinsed with 5 mL of sterile saline and dried with sterile paper points.

Obturation was performed utilizing a modified single cone technique with 4% taper gutta percha cones with auxillary cones, and epoxy resin sealer (Adseal; Meta Biomed). Core-build up composite resin (Ceram X Spercta XT; Dentsply Sirona, Ballaigues, Switzerland) was used to restore the access cavities.

Outcome assessment

Primary outcome:

Post-instrumentation pain was measured at 6-, 12-, 24- , and 48-hours using Numeric Rating Scale (NRS) ranging from (0-10) according to the pain intensity endured. The patient was advised to take an analgesic (400 mg of ibuprofen) at least six hours apart in the case of intense pain.

Secondary outcome:

Microbiological analysis (Intracanal bacterial count)

All root canal samples were collected on 5 ml brain heart infusion broth (Oxoid, Hampshire, UK) and sent to main microbiology department, Cairo University shortly after collection for microbiological analysis. All samples were vortexed for 60 minutes before culture.

Serial 10-fold dilution up to $1/10^4$ were made in brain heart infusion broth to quantify the number of bacteria. 50 µl of undiluted and each of the 4 diluted samples were disseminated on blood and bile esculin agar plates (Oxoid). Blood agar plates were incubated aerobically at 37°C for 24-48 hours (for total aerobic colony count) and bile esculin agar plates were incubated at 37°C in 5% CO_2 for 24-48 hours (for enterococcal colony count).

The resultant growth was quantified in colony forming units/ milliliters (CFU/ml) by counting the colonies on each agar plate and multiplying it by the corresponding dilution factor of each plate.

Enterococci have been identified on blood agar and bile esculin plates using the traditional microbiological techniques based on colony morphology, catalase production, Gram stain, and the black pigmentation on bile esculin agar.

Flare up (interappointment swelling)

Interappointment swelling was recorded as binary data (yes or no) through patient follow-up calls made within 48 hours after instrumentation. If swelling was reported, a clinical examination was arranged to evaluate the condition and to decide whether drainage or oral antibiotics (Augmentin 1 g every 12 hours for 5 days) were required.

Statistical analysis

Data was analyzed using Medcalc software, version 22 for Windows (MedCalc Software Ltd, Ostend, Belgium). Categorical data of pain scores and swelling was expressed as frequency and percentage. Intragroup comparisons

between interventions were done using the Mann-Whitney test, and intragroup comparisons within each intervention were done using the Friedman test, followed by multiple comparisons. Bacterial count and pain intensity data were examined for normality using the Shapiro-Wilk test after logarithmic transformation. Continuous data showed non-parametric distribution and was expressed using median and interquartile range and mean and standard deviation. Intergroup comparison of quantitative values was done using the Mann-Whitney test. Intragroup comparison of quantitative values within each group was done using the Friedman test, followed by multiple comparisons. Relative risk and Hodges-Lehmann median difference were utilized to determine the effect size. The confidence limit was set at 95% with 80% power, statistical significance level of ($p \leq 0.05$), and all tests were two-tailed.

Results

A total of 30 patients were enrolled in this study and divided randomly into 2 groups (n = 15). The flow of participants is represented CONSORT 2025 Flow diagram of the trial design [16] (Fig. 1). Only one patient was lost during follow-up in CH group with a 96.6% retention rate.

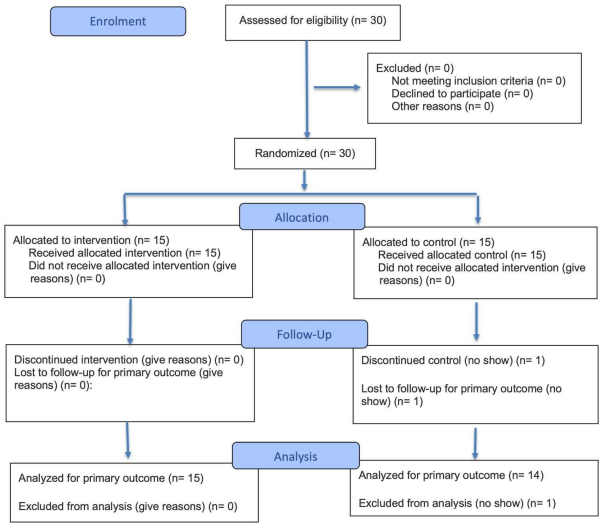

Figure (1): CONSORT 2025 Flow diagram of the trial design

Demographic data

No statistically significant differences were found between the groups in terms of baseline data (age and sex) ($p > 0.05$). Each group has the mean (standard deviation) ages as 43.3 (10.5) and 43.2 (9.6) for the BR and CH groups, respectively. Gender ratio has no significant differences between the two groups, and females were the majority as 86.7% and 66.7% for BR and CH groups, respectively.

Postoperative pain intensity

Intergroup comparison between both groups showed no statistically significant difference at all follow-up periods (6, 12-, 24-, and 48- hours post-instrumentation) ($p > 0.05$). Intragroup comparison within each group showed statistically significant change through time points ($p < 0.00001$; Fig. 2*A*). The median difference in pain intensity was 0 in both groups, with 95% confidence intervals of 0 to 1 for the BR group and 0 to 1.5 for the CH group.

Postoperative pain incidence

Intergroup comparison between both groups showed no statistically significant difference in pain incidence at any follow-up period (6, 12-, 24-, and 48- hours post-instrumentation) ($p > 0.05$). Intragroup analysis within both the BR and CH groups demonstrated significant changes in pain incidence across the time points ($p < 0.00001$, Fig. 2*B*). Success rate was calculated by defining the absence of pain as success and the presence of mild, moderate, or severe pain as failure. After 48 hours, the risk of pain in the BR group was 31% higher compared to the CH group; however, the 95% confidence interval ranged from 0.25 to 6.76 and the difference was not statistically significant ($p = 0.7451$).

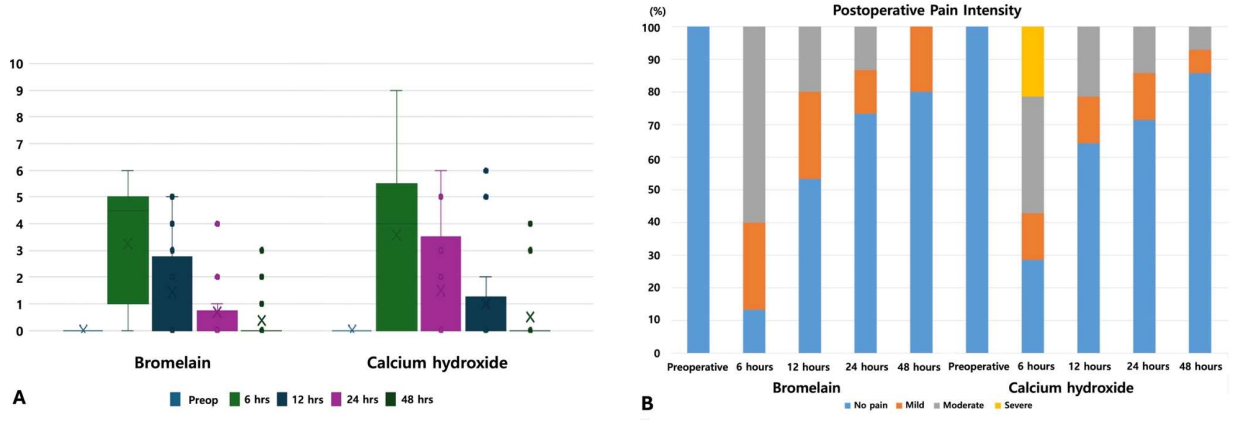

Figure 2. (A) Postoperative pain intensity at different follow-up periods within the Bromelain and calcium hydroxide groups. Box plots show pain intensity scores (NRS) at preoperative, 6, 12, 24, and 48 hours; "×" marks indicate the mean values. No

statistically significant difference was observed between the two materials at any time point, while each group demonstrated significant changes in pain intensity across follow-up periods. (B) Distribution of postoperative pain intensity at different follow-up periods within the Bromelain and Calcium hydroxide groups. Bar segments represent the percentage of patients reporting no pain, mild, moderate, or severe pain at preoperative, 6-, 12-, 24-, and 48-hours post-instrumentation. Both groups showed significant intragroup changes in pain intensity over time, with most patients experiencing no pain by 48 hours. No statistically significant differences were observed between the two groups at any time point.

Bacterial load reduction

Intergroup comparison showed no statistically significant difference in bacterial load between the BR and CH groups in the S3 samples collected after 7 days of medicament application ($p > 0.05$). Intragroup analysis within both BR and CH groups demonstrated significant reductions in bacterial load across the three time points (S1, S2, and S3) ($p < 0.0001$, Fig. 3*A*). The median difference in bacterial load (log CFU/ml) was -2.5 (95% confidence interval -3.00 to -0.50) in the BR group and -3.0 (95% confidence interval -5.00 to -2.50) in the CH group.

Enterococcal count

Intergroup comparison of the enterococcal count in S3 samples revealed no statistically significant difference between the BM and CH groups ($p > 0.05$). Significant decreases in enterococcal counts were observed within both groups across S1, S2, and S3 time points ($p < 0.05$, Fig. 3*B*). The median difference in enterococcal count (log CFU/ml) was -2.5 (95% confidence interval -2.50 to 0.00) in the BM group, while it was 0 (95% confidence interval -2.50 to 0.00) in the CH group.

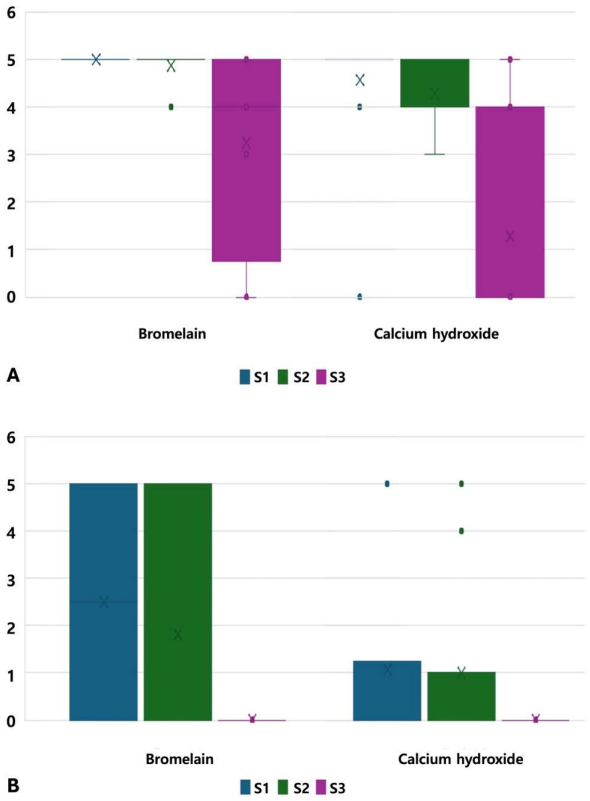

Figure 3. (A) Bacterial load (log CFU/ml) at three sampling time points (S1: baseline, S2: after preparation, S3: after 7 days of medicament application) within the Bromelain (BR) and Calcium hydroxide (CH) groups. Box plots display the distribution of bacterial load, with "×" indicating the mean values. Both groups showed significant reductions in bacterial load across time points ($P < .0001$). No statistically significant difference was observed between the two groups in S3 samples after 7 days. (B) Enterococcal counts (log CFU/ml) at three sampling time points (S1: baseline, S2: after preparation, S3: after 7 days of medicament application) within the Bromelain (BM) and Calcium hydroxide (CH) groups. Box plots display the distribution of enterococcal counts, with "×" indicating mean values. Both groups demonstrated significant reductions in enterococcal counts across time points ($P < .05$), with no statistically significant difference between groups in S3 samples after 7 days.

Incidence of interappointment swelling

The incidence of interappointment swelling was 26.7% in the BM group and 7.1% in the CH group, with no statistically significant difference observed between the two groups ($p = 0.1717$). The relative risk of swelling was 3.7 times higher in the BM group compared to the CH group; however, this difference was not statistically significant (95% confidence interval 0.47 to 29.49, $p = 0.2116$).

Discussion

Most of endodontic patients experience unpleasant post-endodontic pain which vary between 3 - 58% that could lower the patient's quality of life [1]. The most frequent reason for postoperative discomfort is the resilience of infection in the root canal as a result of inadequate canal disinfection [19]. It has been noted that 40% to 60% of root canals still have measurable amounts of bacteria following instrumentation, although chemo-mechanical preparation successfully eliminates a substantial amount of

microorganisms [20]. The use of intracanal medications has proven to eradicate these microorganisms and prevent endodontic flare up owing to their antibacterial properties and ability to effectively relieve discomfort during interappointment [21].

In literature, several intracanal medicaments have been evaluated including calcium hydroxide, chlorhexidine, and antibiotic combinations; however, none fulfill all the ideal requirements of an effective agent, in the term of antimicrobial activity, biocompatibility, and effect on dentin structure [22]. These limitations have encouraged the development of herbal-based alternatives, which may provide safer and more biologically compatible option. Among these natural compounds, bromelain, a proteolytic enzyme complex extracted from *Ananas comosus* (pineapple), exhibits promising antimicrobial, anti-inflammatory, and fibrinolytic effects [10]. Therefore, this study was conducted to evaluate the efficacy of bromelain as an intracanal medicament compared with calcium hydroxide in terms of bacterial reduction, postoperative pain, and interappointment flare-ups in single-rooted lower premolar with necrotic pulps.

In the present study, both bromelain and calcium hydroxide showed no significant difference in incidence and intensity of postoperative pain at any of the assessed follow-up intervals, both groups significantly reduced postoperative pain over time, with pain peaking at six hours and steadily decreasing by 48 hours. This pattern is consistent with the typical postoperative inflammatory response that occurs following canal instrumentation, and the subsequent decline reflects the anti-inflammatory effects of the intracanal medicaments [23]. Bromelain possesses well-documented anti-inflammatory properties, acting by inhibiting prostaglandin synthesis, bradykinin, and pro-inflammatory cytokines while promoting fibrinolysis and reducing tissue edema [24]. These mechanisms likely contributed to the rapid decrease in pain intensity observed in the bromelain group. Similarly, calcium hydroxide's alkaline environment suppresses bacterial endotoxin activity and neutralizes inflammatory mediators [6].

Although, the risk of pain in the BR group was 31% higher compared to the CH group. However, this slight numerical increase was clinically insignificant and may be attributed to transient enzymatic tissue reactions rather than inflammatory exacerbation. Overall, both medicaments were effective in providing patient comfort after treatment.

Regarding intracanal bacterial reduction and enterococcal count, no statistically significant difference was found between both groups in S3 samples (following application of medicament for 7 days), confirming that both medicaments were similarly effective. The antimicrobial action of calcium hydroxide is primarily attributed to its high pH (~12.5), which leads to irreversible damage to bacterial membranes, protein denaturation, and DNA breakdown [6]. In contrast, bromelain, a proteolytic enzyme derived from *Ananas comosus* (pineapple), acts through a distinct non-alkaline biochemical mechanism, The flavonoid constituents of bromelain exhibit a strong affinity for bacterial surface proteins, forming hydrogen bonds that alter cell membrane permeability, leading to protein degradation and biofilm disruption rather than pH-mediated lysis [25,26]. This enzyme-mediated mechanism accounts for bromelain's significant bacterial reduction, equivalent to that of calcium hydroxide, while avoiding the potential adverse effects of a highly alkaline environment on dentinal integrity.

However, these findings conflicted with those of other researchers [14,15] who concluded that bromelain has superior antimicrobial properties than calcium hydroxide paste. No significant difference between the two intracanal medicaments in this study can be due to the inherent weakness in the culture method, as not all viable bacteria can be recovered or cultured, grading it as a lower sensitivity [27]. However, it is still an excellent choice for providing a broad range of information regards the endodontic microbiota and a quick method for evaluating the clinical efficacy of a novel technique or intervention. Another possible reason for this variation is the limited sample size.

Regarding interappointment flare-up, BR has shown 26.7% (4 out of 15 patients) interappointment swelling, while CH has shown 7.1% (1 out of 14 patients), with no statistically significant difference between them. Although the relative risk suggested a higher likelihood of swelling with bromelain (RR = 3.7), the wide confidence interval (0.47–29.49) and lack of significance indicate that this difference is clinically negligible. The mild and transient swelling observed in a few bromelain-treated cases may be explained by its proteolytic enzymatic activity, which can temporarily increase vascular permeability and trigger a short-lived inflammatory response during early periapical healing [28]. This mild reaction was self-limiting and did not constitute a true flare-up. Conversely, calcium hydroxide's highly alkaline environment likely contributed to its lower swelling rate by neutralizing bacterial endotoxins and inflammatory mediators [6].

In this study, anaerobic culture was not performed due to its technical difficulties, such as the requirement for specialized anaerobic chambers, strict environmental control, and careful sample handling to prevent oxygen exposure. These procedures are time-consuming, technique-sensitive, and resource-intensive, which often limit their practical use in clinical studies [29]. To partially overcome this limitation, the study evaluated facultative anaerobes, particularly *Enterococcus* species, which are commonly found in persistent endodontic infections and can survive under both aerobic and anaerobic conditions [30]. Their inclusion helped in assessing intracanal disinfection efficacy despite methodological constraints.

In addition, molecular microbiological techniques were not used because of their high cost, technical complexity, and inability to differentiate between viable and non-viable microorganisms. While these methods provide higher sensitivity and allow detection of a wide range of bacterial species, they may lead to overestimation of bacterial presence and require advanced laboratory facilities that are not routinely available [31].

Conclusion

Within the limitations of this study, the findings provide preliminary evidence supporting the potential of bromelain as an intracanal medicament for reducing intracanal bacterial load, severity of postoperative pain, and incidence of interappointment swelling. However, further clinical trials with larger sample size are required to confirm its

clinical effectiveness and safety relative to calcium hydroxide.

Authors' contributions

Conceptualization: M.E.E. and M.M.A.; Methodology: M.E.E. and S.T.S.; Formal analysis: M.E.E. and M.M.A.; Funding Acquisition: M.E.E. and M.M.A.; Resources: A.M. and M.M.A.; Data Curation: M.E.E. and M.M.A.; Writing: Original Draft: M.E.E. and M.M.A.; Writing: Review & Editing: M.M.A.

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Conflict of interest

The authors declare no competing interests.

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Declarations

Ethics approval and consent to participate

The study protocol was registered at www.clinicaltrials.gov (ID: NCT05687760) after its approval by the institutional review board/ethical committee in the Faculty of Dentistry - Cairo University (approval number 1223). Informed consent was obtained from all participants after explaining the study objectives and procedures prior to their enrollment.

Data availability

The data presented in this study are available on request from the corresponding author.

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Figure legends

Figure 1. CONSORT 2025 Flow diagram of the trial design.

Figure 2. (A) Postoperative pain intensity at different follow-up periods within the Bromelain and calcium hydroxide groups. Box plots show pain intensity scores (NRS) at preoperative, 6, 12, 24, and 48 hours; "×" marks indicate the mean values. No statistically significant difference was observed between the two materials at any time point, while each group demonstrated significant changes in pain intensity across follow-up periods. (B) Distribution of postoperative pain intensity at different follow-up periods within the Bromelain and Calcium hydroxide groups. Bar segments represent the percentage of patients reporting no pain, mild, moderate, or severe pain at preoperative, 6-, 12-, 24-, and 48-hours post-instrumentation. Both groups showed significant intragroup changes in pain intensity over time, with most patients experiencing no pain by 48 hours. No statistically significant differences were observed between the two groups at any time point.

Figure 3. (A) Bacterial load (log CFU/ml) at three sampling time points (S1: baseline, S2: after preparation, S3: after 7 days of medicament application) within the Bromelain (BR) and Calcium hydroxide (CH) groups. Box plots display the distribution of bacterial load, with "×" indicating the mean values. Both groups showed significant reductions in bacterial load across time points ($P < .0001$). No statistically significant difference was observed between the two groups in S3 samples after 7 days. (B) Enterococcal counts (log CFU/ml) at three sampling time points (S1: baseline, S2: after preparation, S3: after 7 days of medicament application) within the Bromelain (BM) and Calcium hydroxide (CH) groups. Box plots display the distribution of enterococcal counts, with "×" indicating mean values. Both groups demonstrated significant reductions in enterococcal counts across time points ($P < .05$), with no statistically significant difference between groups in S3 samples after 7 days.