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EFFECT OF PROPOLIS NANOPARTICLES VERSUS SODIUM HYPOCHLORITE AS ROOT CANAL IRRIGANT ON POSTOPERATIVE PAIN AND BACTERIAL REDUCTION IN MANDIBULAR PREMOLARS WITH NECROTIC PULPS: A RANDOMIZED CLINICAL TRIAL

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ABSTRACT

Aim: The aim of this study was to clinically compare the intensity of post-operative pain and the amount of bacterial load reduction after using 20mg/ml propolis nanoparticles extract solution as root canal irrigant versus 2.5% NaOCl during chemo-mechanical preparation in asymptomatic necrotic mandibular premolars treated in a single visit.

Methodology: This is a double blinded, parallel, prospective randomized controlled trial, with allocation ratio 1:1. Fifty patients having single canalled necrotic mandibular premolar were included. Intervention group received 20 mg/ml propolis nanoparticles solution as irrigant, while control group used 2.5% NaOCl. Single visit endodontic treatment was done using EdgeEndo files. Postoperative pain was measured at 6, 12, 24, and 48 hours using Numerical Rating Scale. Culturing was used to assess intracanal bacterial levels before and after preparation. Also, the number of analgesic tablets were counted. All demographic, baseline and outcome data were statistically analyzed. A *P*-value < 0.05 was considered statistically significant.

Results: Postoperative pain in both groups was similar regarding incidence and intensity (*P*>0.05). No significant difference in the incidence or the number of analgesic tablets taken by the patients was detected in both groups (*P*>0.05). Both irrigants significantly reduced the intracanal bacterial load however, propolis nanoparticles solution showed superior efficacy against anaerobes (*P* = 0.027).

Conclusion: Propolis nanoparticles solution was similar to NaOCl regarding incidence and intensity of postoperative pain but showed higher anaerobic bacterial reduction when treating asymptomatic necrotic mandibular premolars in a single visit.

KEYWORDS: Bacterial reduction, propolis nanoparticles (PN), post-operative pain, endodontic irrigants, sodium hypochlorite (NaOCl).

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INTRODUCTION

Postoperative endodontic pain is an unpleasant occurrence influencing the patient's quality of life. Its incidence ranges from 3% to 58% [1,2]. Microorganisms together with their by-products present in the infected root canals, are regarded as major factors causing postoperative pain and/or the development of periapical inflammation [1,3,4].

Chemo-mechanical preparation is of outmost importance to reduce bacterial population in the root canal. Effective disinfection cannot be achieved through mechanical instrumentation alone as the complex root canal anatomy prevents optimal accessibility for the instruments and serves as a shelter for microorganisms [5].

Sodium hypochlorite (NaOCl) is the most popular irrigant in endodontics because of its broad antibacterial spectrum and high ability to dissolve pulp tissues. However, it is regarded as a potential irritant for periradicular tissues especially when used in high concentrations [6]. All these demerits prompt the search for a more biocompatible alternative.

Recently, herbal products gained research interest worldwide because of their medicinal properties including high biocompatibility, anti-microbial, and anti-inflammatory properties favoring their use as root canal irrigants [7,8]. One of these herbal products is propolis. Propolis meaning the "city's guardian", other references call it Russian Penicillin [9], is a natural resinous substance made by the honeybee. Its major chemical constituents are flavonoids, phenolics, and several aromatic compounds [10]. Flavonoids are popular plant compounds having antioxidant, anti-inflammatory, antibacterial, and antifungal properties.

Additionally, nanotechnology has revolutionized in several aspects of the medical field. Anti-microbial nanoparticles offer various advantages including the increased surface area to volume ratio, their ultra-small sizes, together with their superior physical and chemical properties [11]. Thus, nanoparticles can enhance the properties of antimicrobial agents by improving their effectiveness, accuracy, and speed of action. Using nanoparticles to combat infections of the root canals is of a significant importance as they have anti-adhesive, and excellent delivery capabilities [12].

Results of available in vitro studies showed that utilizing herbal irrigants is promising [13,14]. However, no evidence-based investigations have evaluated the intensity of postoperative pain and the amount of bacterial load reduction when propolis nanoparticles were used as an irrigant in teeth with necrotic pulpal tissues. So the research question was to test whether the use of 20 mg/ml propolis nanoparticles extract solution as an endodontic irrigant rather than 2.5% NaOCl will show difference in postoperative pain and in the amount of bacterial reduction in patients with asymptomatic necrotic mandibular permanent premolars.

MATERIALS AND METHODS

Study design, settings and sampling

The study has been designed as a parallel, prospective, double-blinded, RCT with an allocation ratio 1:1 and framework superiority. The protocol was registered at www.clinicaltrials.gov (ID: NCT05146713) and it was approved by ethics committee at Faculty of dentistry, Cairo University (Approval Number 5-11-21).

Eligibility criteria

The inclusion criteria were patients aged from 20 to 55 years having asymptomatic mandibular premolar teeth diagnosed with pulp necrosis with or without apical periodontitis.

Patients who consumed analgesics or antibiotics during the past 12 hours, pregnant women, teeth associated with acute periapical abscess or acute exacerbation of a chronic abscess, and previously accessed teeth were excluded from the study.

The final diagnosis was confirmed through the history of chief complaint reporting no pain with hot and/or cold stimuli, negative response to both thermal test using a hot instrument and electric pulp tester (China), and radiographic examination using Digora intraoral periapical sensor plate and software (Finland) showing mandibular premolar with (smaller than 3 mm) or without periapical radiolucency.

Sample size calculation

It was calculated from a previous study by Hosny et al., 2021 [7]. Using power 80% and 5% significance level a total of 22 patients in each group (total 44 patients in the two groups) were studied. To compensate for losses during follow-up, the number increased to 25 per group (drop-out rate 15%).

Randomization and blinding

A computer software (http://www.random.org/) was used for generation of a random sequence from (1-50) arranged into two columns. The table was kept with the co-supervisor (R.S.). Eight folded numbered papers were to be dragged by the patients to be allocated to either group based on this number.

Preparation of propolis nanoparticles extract solution

Raw propolis powder was brought from Imtenan health shop (Imtenan health shop, Obour City, Egypt) then transferred to nanotech company (Nanotech Egypt, Giza) to produce propolis nanoparticles solution. Raw propolis particles were made in the nano-scale using liquid anti-solvent precipitation (LAP) technique [15,16] to produce ultra-fine particles having size ranging from 12 to 18 nm and spherical shape ((Table 1) and Figure (1)). Hydrophilic and biocompatible polymer (polyethylene glycol (PEG)) was used to synthetize propolis nanoparticles solution. By dissolving the required amount of nanoparticles and using ultrasonication, propolis nanoparticles solution was synthesized. PEG is totally inert, having no antibacterial action so only the antibacterial action of propolis was tested throughout the study.

Preoperative pain assessment

A Numerical rating scale (NRS) was given to each patient to choose a mark from 1-10. Pain levels were categorized into 4 categories where: 0 = no pain, (1–3) mild pain, (4–6) moderate pain and (7– 10) severe pain.

TABLE (1) Properties of propolis nanoparticles

Appearance (color)	Concentration	Size (TEM)	Shape (TEM)
Dark brown/ black	20 mg/ml	15 ± 3 nm	Spherical like shape

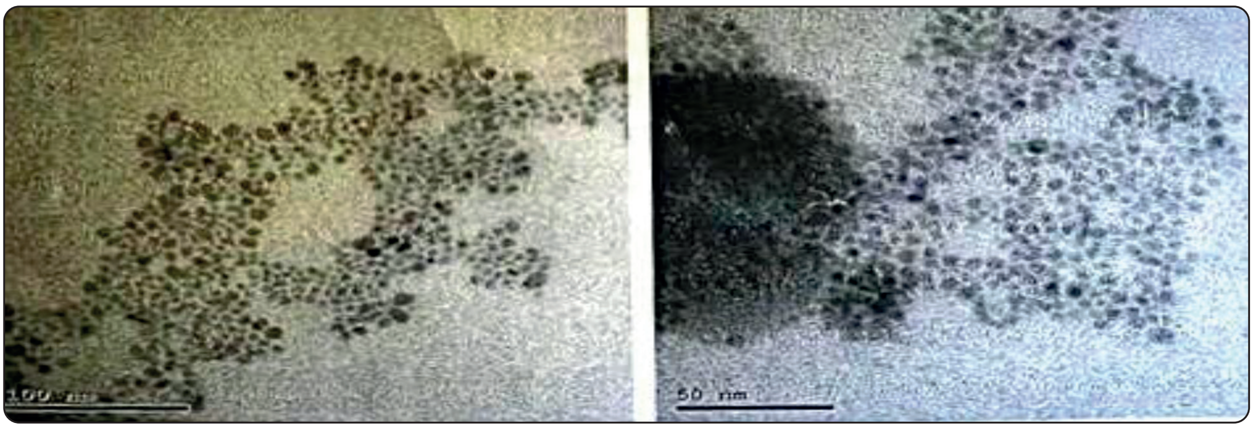

Fig. (1) Transmission electron microscope picture of propolis nanoparticles

Root canal treatment procedures and sampling

Single-visit endodontic treatment was accomplished, after signing an informed consent by the patients. Following administration of 1.8 mL of 2% Mepivacaine HCl with Levonordefrin 1:100,000 (Alexandria, Egypt), isolation of the operational field was accomplished then its disinfection using 3% H_2O_2 followed by 5.25% NaOCl. Preparation of the access cavity was done with round bur & Endo-z bur (Dentsply Maillefer, Ballaigues, Switzerland). Disinfection of the operative field was done once more then NaOCl was neutralized using 5% sodium thiosulfate. Sterility checking was done through culturing of paper point samples taken from the access cavity and the external crown surface. Positive sample indicate a breach in the aseptic conditions so, the case was excluded from the study.

Regarding the pre-instrumentation bacterial sample (S1), sterile paper points were inserted and left to absorb the fluid for 60 seconds, before being transferred to tubes containing 2 ml of sterile brain heart infusion broth (England).

Working length was determined using electronic apex locator (Root ZX, J. Morita). Mechanical preparation was done using EdgeEndo file system (Albuquerque, NM, USA) in continuous rotary brushing motion; 3 rotary files having 4% taper were used reaching an apical size preparation of 35 or 40 according to the size of the canal, and ensuring the establishment of definite apical stop. Thorough irrigation was done using plastic disposable syringe with a needle gauge 30 (Korea) 1 mm from the working length. The same irrigation volume was received by all teeth (5 ml before instrumentation, 5 ml between each file and 5 ml as a final flush reaching total volume of 25 ml). After mechanical instrumentation, manual dynamic agitation was done using a well-fitted master cone in a push pull strokes.

Sodium thiosulfate was used to inactivate NaOCl and the canals were flushed with sterile saline then 17% EDTA solution (India) for 1 min. The post-instrumentation sample (S2) was collected as mentioned. Both microbiological samples were transferred for culturing within 20 minutes. Modified single cone technique with a resin-based root canal sealer (AdSeal) was used for obturation and a temporary restoration (Cavit, Germany) was utilized to seal the access cavity.

Postoperative pain assessment

Both incidence & severity of the postoperative pain were recorded using NRS at 6, 12 hours, 1 day and 2 days after obturation. Ibuprofen (400mg) (Novartis, Egypt) was prescribed if patient felt moderate or severe pain.

Intracanal bacterial count

Culture technique was used for the microbiological analysis. Samples in 1 ml of BHI broth were dispersed with vortex in the mixer for 60 secs. Then preparation of serial 10 folds dilution (1/10 and 1/100) was made. For aerobic bacterial culture, 50 µl were cultured over BHI agar plates and incubated at 37ºC for 24 hours. Regarding anaerobic bacteria, another 50 µl were cultured on BHI agar plates, were placed in a sealed anaerobic jar containing GasPak & an anaerobic indicator (England) then incubated at 37ºC for 48 hours.

Visual quantification of the resultant growth was made under the microscope through counting the number of colony forming units per ml (CFUs/ml) of each dilution [17], then multiplied by the dilution factor to obtain the actual bacterial count. Log bacterial load reduction was calculated using the equation: Log bacterial load reduction = log10*(Mean CFU at S1 / Mean CFU at S2) [18].

Statistical analysis

All data were collected and statistical analysis was performed by IBM (IBM Corporation, NY, USA) and Statistical Package for Social Science (SPSS) version 22 for windows (SPSS, Inc., Chicago, IL, USA). Data were tested for normality using

Shapiro Wilk test. Continuous data was presented as mean, standard deviation (SD). Independent t-test was used for nonrelated samples comparisons of normally distributed data, while Mann – Whitney U test was used for non-related samples comparisons of non-normally distributed data. Wilcoxon signed rank test was used for two related samples comparisons, while Friedman's test was used for more than two related samples comparisons followed by Dunn's post hoc test for pairwise comparisons. Categorical data was presented as frequencies (N) and percentages (%) and were analyzed using Chi square test or Fisher exact test. Significance level for primary tests was set at $P < 0.05$.

RESULTS

65 patients were assessed for meeting the eligibility criteria, 50 patients were enrolled and included in the analysis. Patients enrollment and flow through all study phases is demonstrated in figure 2. Demographic data are presented in table 2. No significant difference was detected between the two groups regarding gender distribution (*P*= 0.771), age (*P*= 0.986), tooth type (*P*= 0.544), and presence of preoperative pain (*P* = 1).

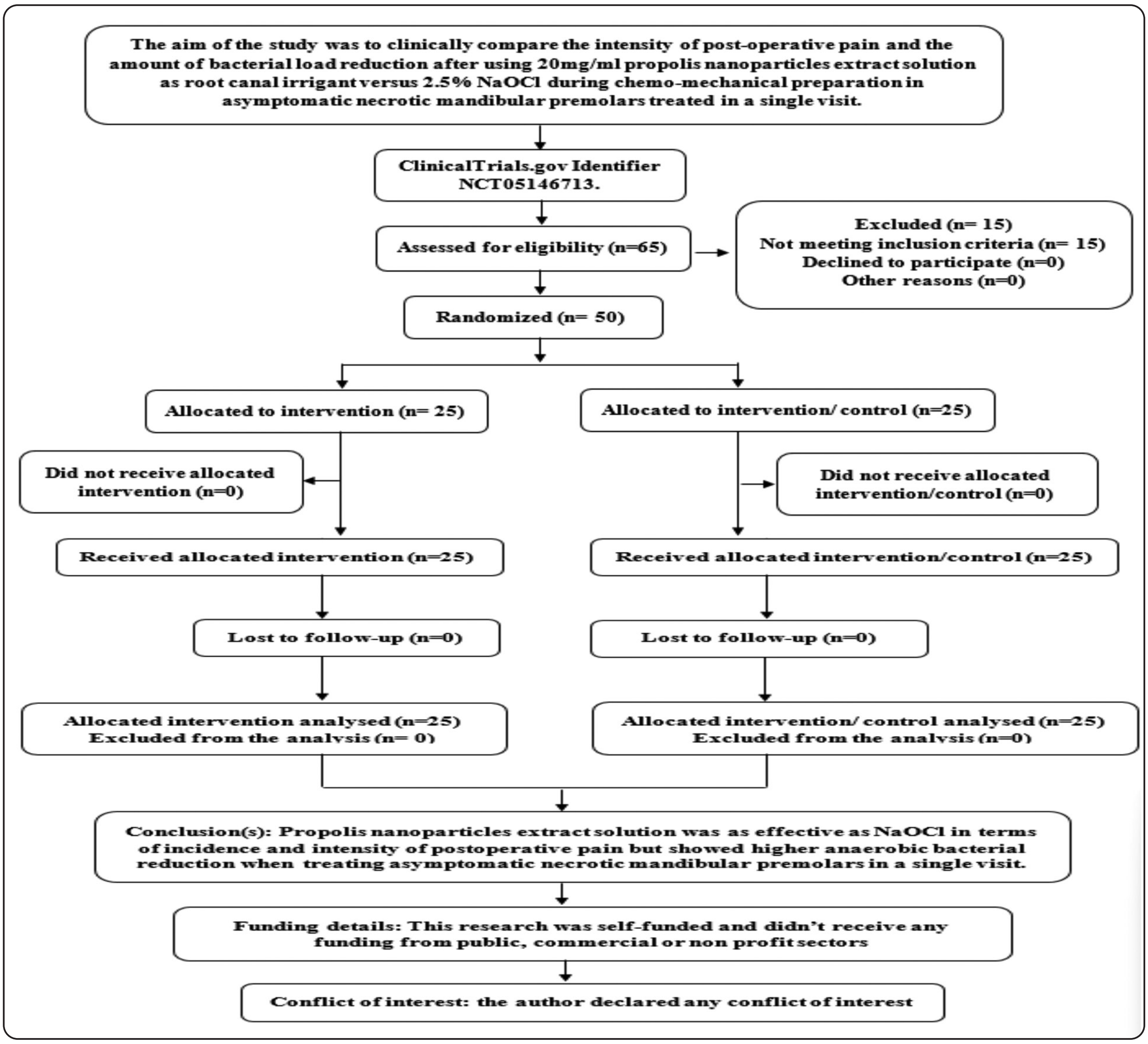

Fig. (2) PRIRATE 2020 flow chart

TABLE (2) Demographic baseline data

	Control group n - 25	Intervention group n - 25	*P* value
Age (in years)			
Mean (SD)	31.6 (8.2)	31.6 (7.9)	0.986[N.S]
Gender			
Male	9 (36%)	10 (40%)	
Female	16 (64%)	15 (60%)	0.771[N.S]
Tooth type (n (%))			
Lower 4	9 (36%)	7 (28%)	
Lower 5	16 (64%)	18 (72%)	0.544[N.S]
Pre-operative pain			
Mean (SD)	0 (0%)	0 (0%)	1

N.S: non-significant (p>0.05)

The incidence of postoperative pain for both groups is reported in figure 3. No significant difference was detected between the two groups regarding incidence of postoperative pain at all-time intervals (*P*>0.05). Postoperative pain intensity increased in both groups at 6 hours then decreased substantially over time reaching its lowest at 48 hours without significant difference between both groups (*P*>0.05) (figure 4).

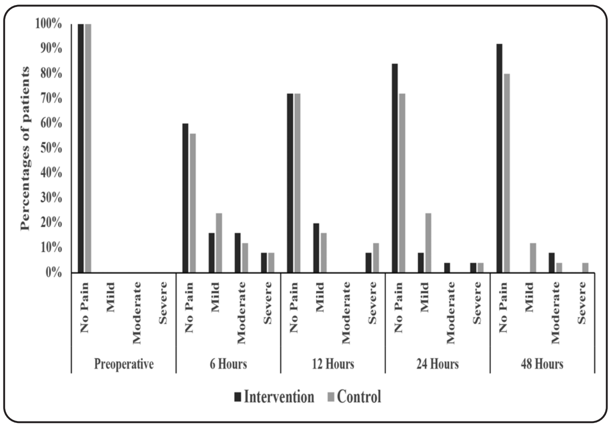

Fig. (3): Bar chart representing the incidence of pain categories at different time intervals for both group

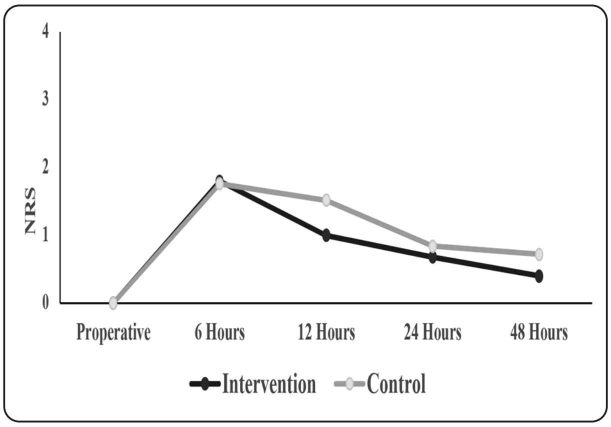

Fig. (4) Line chart representing the changes in pain intensity at different time intervals for both groups.

Following obturation, within the intervention group, 16% of patients reported taking analgesics, while within the control group, 20% of patients reported taking analgesics without significant difference between the 2 groups.

No bacterial growth was detected in all sterility control samples, therefore, all cases were included in the analysis. Mean and standard deviation (SD) values of Log bacterial count reduction between both groups are presented in table 3. Both irrigants showed considerable bacterial load reduction following chemo-mechanical preparation. Propolis nanoparticles solution significantly reduced anaerobic bacterial load than NaOCl (*P* = 0.027)), while no significant difference was detected between the two irrigants regarding both aerobic (*P*= 0.937) and total bacterial load reduction (*P*= 0.211).

TABLE (3) Log bacterial load reduction between the two groups

	Intervention	Control	*P* value
Log bacterial load reduction (mean (SD))			
Aerobic	1.02 (0.28)	1.08 (0.41)	0.937
Anaerobic	1.08 (0.41)	0.98 (0.24)	0.027*
Total	1.05 (0.34)	1.10 (0.33)	0.211

Significant at P <0.05

DISCUSSION

Propolis, for many years, has been recognized for its anti-inflammatory, anti-bacterial, anti-viral, and anti-fungal activity. Furthermore, nanoscale systems have been utilized to enhance the biological and physical properties of antimicrobial agents used in endodontics thereby improving their effectiveness in root canal debridement [3]. In this context, previous nano-herbal irrigants showed promising results regarding antibacterial and anti-biofilm activity in vitro [19-23]. Therefore, this study assessed the effect of 20mg/ml propolis nanoparticles extract solution versus 2.5% NaOCl as root canal irrigant on the intensity of postoperative pain, the amount of bacterial reduction, and analgesic intake following single visit root canal treatment of asymptomatic mandibular premolars with necrotic pulps in a prospective, randomized, double-blinded, parallel clinical trial.

Fifty patients having asymptomatic single canalled necrotic mandibular premolar were included. Only asymptomatic patients were included in this study as pre-operative pain has been reported to be significant predictor for post-treatment pain [24]. Teeth having large periapical lesions have been excluded because this indicates long-standing infections, cystic transformation or extraradicular infection negatively affecting the outcome [25,26].

Single visit approach was adopted in this investigation. A well conducted systematic review stated that single visit approach causes less postoperative complications by reducing mechanical and chemical injuries to the periradicular tissues and omitting the risk of inter-appointment leakage[27]. Additionally, both single-visit and multiple-visit endodontic treatment demonstrated comparable success rates.

2.5% NaOCl was used as it has less cytotoxicity than 5.25% NaOCl. Furthermore, previous studies showed that low NaOCl concentrations caused less postoperative discomfort than higher concentrations[1,28].

The current study used 20 mg/ml propolis nanoparticles (PN) extract solution. After being prepared in the nano-scale, the raw propolis particles were dissolved in PEG which is a hydrophilic biocompatible polymer[8]. PEG was selected as it is totally inert thus, eliminating any confounders and ensuring that only the antibacterial action of PN is being tested. It was shown that ethanolic extract of propolis demonstrated added antibacterial effect to propolis [29].

Rotary instrumentation was done using EdgeEndo files. The files are manufactured from annealed heat-treated nickel titanium alloy called Fire-Wire, having controlled memory, centering ability and high flexibility [30]. The manufacturer claims that the variable pitch maximizes the cutting efficiency and removes the debris in a coronal direction reducing their extrusion apically and thus less postoperative pain.NRS was used in this study, as it offers high patient compliance and applicability [31], and has been utilized for outcome assessment in various studies [1,7,32].

A follow up period of 48 hours was selected as pain prevalence decreases substantially during the first 2 days following obturation [2]. Pain assessment began at 6 hours postoperatively, to be sure that the local anesthetic effect was totally worn off, then continued to be assessed at 12, 24, and 48 hours postoperatively [33].

Statistical analysis demonstrated that the two groups exhibited comparable baseline data indicating successful randomization. Both incidence and severity of postoperative endodontic pain did not significantly differ between both groups at all-time periods. At 6 hours, the highest level of mean pain score was observed and then decreased gradually reaching its lowest at 48 hours. Intervention group showed less mean pain score at 12 hours, 1 day and 2 days postoperatively than control group but it did not reach statistical significance level. No clinical studies investigated the effect of PN solution as an irrigant on post-endodontic pain to date. However, the results of this investigation were in agreement with another study founding that postoperative pain after using neem (Azadirachta indica), another herbal irrigant, was similar to 2.5% NaOCl at all the follow-up periods except at 24 hours where neem caused lower pain intensity [7]. Also, our results were in conjunction with another study which showed no significant difference in the postoperative pain following the use of nano-silver irrigant or NaOCl during chemo-mechanical preparation of asymptomatic necrotic teeth [34].

The rationale for the lower pain values after using propolis nanoparticles solution could be associated with the anti-inflammatory and antibacterial effects of propolis. Additionally, propolis nanoparticles solution exhibits better anti-adherence and anti-biofilm activity than propolis due to its better penetration capabilities [35]. The anti-inflammatory effect of propolis is mainly related to its high content of flavonoids & the presence of caffeic acid phenylethyl ester (CAPE). Flavonoids inhibit the synthesis of glycoxygenase, lipoxygenase, prostaglandin, nitric oxide, and protein kinases [9]. Additionally, CAPE hinders lipoxygenase formation from arachidonic acid thus, reduces the inflammation. Furthermore, CAPE increases IL4 and IL10 production which are potent anti-inflammatory cytokines [36]. CAPE also inhibits the activation of the nuclear transcription factor NF-Kappa B by pro-inflammatory agents including tumour necrosis factor (TNF). This is attributed to the inhibition of reactive oxygen species which are crucial for NF-Kappa B activation [37].

Biofilm sampling using sterile paper points has several limitations; it is time consuming, and the presence of uncultivable bacteria. However, culturing indicates treatment effectiveness and allows quick evaluation of its outcome rather than waiting for yearly follow-up radiographs [38,39]. It is considered the gold standard technique and it is a well-established protocol in several RCTs [24,40,41].

The antibacterial efficacy of propolis nanoparticles could be attributed to several mechanisms. The active constituents of propolis attach to the cytoplasmic membrane causing cell death by 2 ways. First, propolis reduces ATP production compromising bacterial mobility. The second way is by perforating the membrane so the cytoplasmic content is expelled outside causing cellular death. Another mechanism of action could be explained by its high flavonoid content. Flavonoids inhibit topoisomerase IV-dependent inhibiting bacterial growth[5]. Furthermore, propolis inhibits nucleic acid synthesis through inhibiting bacterial DNA dependent RNA polymerase [42]. Additionally, quercetin, the predominant flavonoid in propolis, binds to DNA gyrase inhibiting ATPase activity [43].

The mean reduction in CFUs/ml was similar regarding aerobic and total bacterial counts in both groups, however, propolis nanoparticles solution significantly decreased the anaerobic bacterial load compared to NaOCl. This is in contrast to the results of a RCT showing similar reduction in the anaerobic bacterial counts after using 20% propolis, 3% NaOCl or 2% CHX [18]. Similarly, the findings of this investigation are in contrast to another study demonstrating that 2% CHX & 5% NaOCl had superior anti-microbial effectiveness than 10% propolis and 100% morinda citrifolia [44]. Also, Jaiswal *et al.*, (2017) reported that propolis exhibited similar antimicrobial efficacy to NaOCl [45]. The fact that these studies used propolis while this investigation tested the effect of propolis nanoparticles, may account for the differences in results.

The findings of this study may be corroborated to those reported by an in vitro study showing that 300 μg/ml propolis nanoparticles solution had greater anti-bacterial efficacy than propolis, 2% CHX & 6% NaOCl [23]. This is also in accordance with the data reported by Madani et al., (2022) who demonstrated that propolis nanoparticles solution with a concentration 10 folds less than propolis exhibited superior anti-microbial effectiveness [35]. However, another investigation did not show significant difference in CFUs count following chemo-mechanical preparation using either 5.25% NaOCl, nano-propolis or nano-chitosan [21]. The scientific explanation behind the agreement in the results could be related to the use of propolis nanoparticles. Nanomaterials have unique properties in terms of their increased chemical reactivity and large surface to core ratio as opposed to their bulk counterparts [46]. Being positively charged, nanoparticles effectively bind to the negatively-charged cell membrane causing marked increase in membrane permeability so, allows more nanoparticles to enter inside the bacterial cell, thus causing leakage of the cellular content. The nanoparticles also bind to mesosomes, affecting the cell respiration, division and DNA replication. Another antibacterial mechanism of nanoparticle could be explained by the release of ROS causing oxidative stress in the cell and reduces ATP production leading to membrane disruption and cell lysis [11].

The clinical outcomes of using propolis nanoparticles solution as root canal irrigant seem promising. Further studies are required to test propolis nanoparticles solution with other concentrations to evaluate its effectiveness on bacterial load reduction and the amount of endotoxins to determine if it could be an alternative to NaOCl in endodontic treatment.

CONCLUSION

Within this study limitations, it can be concluded that propolis nanoparticles solution could be as effective as NaOCl with no increase in the incidence or intensity of postoperative pain. Both irrigating solutions significantly reduced intracanal bacterial levels but propolis nanoparticles solution showed superior effectiveness against anaerobic bacteria in primary infected root canals treated in a single visit.

Conflict of interest

The authors stated that there are no conflicts of interest related to this article.

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