EGYPTIAN DENTAL JOURNAL
VOL. 66, 2815:2824, OCTOBER, 2020
PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360
CONSERVATIVE DENTISTRY AND ENDODONTICS
www.eda-egypt.org • Codex : 115/2010 • DOI : 10.21608/edj.2020.41856.1245

EVALUATION OF THE ANTIBACTERIAL EFFICACY OF NEWLY FORMULATED NANO TRIPLE ANTIBIOTIC ASTE WITH NANO ANTI-INFLAMMATORY DRUG AS A ROOT CANAL MEDICAMENT. (A DOUBLE BLIND RANDOMIZED CLINICAL TRIAL)

Yousra Mohamed Nashaat*

ABSTRACT

Intracanal microorganisms' elimination is essential for root canal treatment long-term success. Proper mechanical preparation and irrigation in conjugation with intracanal medicaments can achieve this goal. The aim of this study was to evaluate the ability of Nano triple antibiotic paste with Nano diclofenac potassium anti-inflammatory drug (Nano TAPC) versus regular Triple antibiotic paste with diclofenac potassium anti-inflammatory drug (TAPC) and Calcium hydroxide (Ca (OH)2) used as intra-canal medication in reducing intracanal bacterial count.

Methods: 60 patients with upper central incisors were chosen (all necrotic and asymptomatic) and then they were divided into three groups according to the intra-canal medication used: Ca (OH) 2, TAPC and Nano TAPC. Access cavity was performed for the targeted teeth followed by root canals preparation using rotary Protaper Next files with saline irrigation. Bacteriological samples were obtained from the root canals before (S1) and after instrumentation (S2) in the first treatment session. Subsequently, intracanal medication was placed and bacterial reduction was assessed in the second session after 3 days (S3) using colony forming unit test.

Results: There was a statistically significant bacterial count reduction in all groups from S2 to S3. Nano TAPC intracanal medication achieved significant bacterial count reduction compared to that of TAPC intracanal medication and Ca (OH) 2.

Conclusion: NTAPC as intracanal medication was more effective in reducing the root canal total bacterial count than that of the TAP and that of the Ca (OH) 2 in asymptomatic uniradicular necrotic teeth.

KEYWORDS: Anti-inflammatory, Bacterial count, Calcium Hydroxide, Nano TAPC, TAPC.

* Associate Professor of Endodontics, Endodontic Department, Faculty of Dentistry, October 6 University, Cairo, Egypt.

INTRODUCTION

Systemic antibiotics were effectively used to control root canal infections in conjugation with endodontic treatment but their administration sometimes cause some adverse effects, moreover they depend on the circulatory system to distribute the drug to the infected site. Since the necrotic pulpless teeth are deprived from the normal blood supply so it becomes impossible for the drug to reach its target area, therefore, antibiotics' local application within the root canal system may be a more efficient for delivering the drug [(1)].

Root canal infections are polymicrobial, aerobic and anaerobic species both exists which makes the usage of a single antibiotic not enough for complete microbial eradication. Therefore, combination of antibiotics, mainly consisting of ciprofloxacin, metronidazole and minocycline, referred to as triple antibiotic (TA) paste has been suggested for root canal disinfection. [(2)].

Nowadays, -Nanotechnology- term firstly used by Prof. Kerie E. Drexeler in 1980's- is progressing as a promising field that is developing every day in various biomedical applications. The purpose of Nano dentistry is to maintain the near-perfect oral health through the use of nano materials including tissue engineering and nano robotics. Antibacterial activity of Nano materials have gained popularity as a result of their broad spectrum of activity and biocompatibility [(3)].

MATERIALS AND METHODS

The Research Ethics Committee of Faculty of Dentistry, October 6 University. (No.6-2019) approved this study. All the clinical procedures were undertaken with the understanding and written consent of each subject before treatment sessions. The article was written in concordance with the CONSORT checklist 2010.

Study setting: The study design is a parallel double blind randomized controlled clinical trial with allocation ratio 1:1 and a Superiority Framework. The study was conducted in the duration between June 2019 and March 2020 in the endodontic clinic Faculty of Dentistry, October 6 University.

Sample size: A total sample size of 42 case patients and 42 control patients was sufficient to reject the null hypothesis that the exposure rates for case and controls were equal with probability (power) 0.8 as the true probability of success among intervention group is 0.65. The Type I error probability associated with this test of this null hypothesis was 0.05. The sample size was calculated by G power program (University of Düsseldorf, Düsseldorf, Germany).

Participants' inclusion criteria: both male and female patients with age range 20-40 years, medically free with maxillary asymptomatic necrotic central incisors with mature closed apex.

Exclusion criteria: patients above 40 years, premedicated patients or patients with dental related symptoms and those chronic diseases.

Preoperative x-ray was taken for each targeted tooth, administration of local anesthesia and preparation of access cavities was done. After application of rubber dam for isolation; the canals patency were checked using size 15 K type file and working length was determined.

The canals were enlarged to size 25 K-type file with no irrigation. Then each canal was filled with sterile distilled water using a sterile plastic syringe to obtain the first pre-chemo mechanical bacteriological sample (S1). Then a 3 sterile paper points size 25 were placed in the canal for 1 minute. The paper points were immediately transferred into test tube with thioglycolate.

Laboratory procedures: Samples were immediately processed in the laboratory inside an anaerobic chamber (85% N2, 10% H2 and 5% CO2) (Coy Laboratory Products, Grass Lake, MI, USA). The entire 4-mL sample was vortexed for 1 minute.

An aliquot of 1 mL was then diluted in enriched thioglycolate broth using 10-fold serial dilutions until 10-4. Each diluted sample was inoculated on a 5% sheep blood CDC anaerobic agar plate with vitamin K and hemin (BBL Becton Dickinson de Mexico) and was incubated at 37°C for 2-7 days. Colony-forming units (CFU) for bacteria were counted using pour plate method [4].

Mechanical preparation was done using rotary ProTaper Next instruments (Dentsply Maillefer). Canals were irrigated with 2ml of sterile physiologic saline between each instrument and the next till file X4 (40/06). The second bacteriological sample (S2) (Post-chemo mechanical) was acquired with the same technique like that done in the first bacteriological sample.

Laboratory procedures were the same like that done in the first bacteriological sample (2).

Patients were randomly divided using computer generated randomization (www.random.org) into three groups (n=20/group) according to the intracanal medication to be used:

1) ***Group A (Control group) :*** Calcium hydroxide paste (Ca (OH) 2) (Metapex, META BIOMED Co,).

2) ***Group B:*** Triple antibiotic paste + Catafast (TAPC).

TAPC consists of: Ciprofloxacin (Ciprofloxacin 500 mg; Amriya pharm, Alexandria, Egypt), Metronidazole (Flagyl 500 mg; Sanofi Aventis, Cairo, Egypt) and Minocycline (Minocin 50 mg: Sedico, Giza, Egypt). In addition to Catafast which consists of 50 mg NSAIDs granules for oral solution (Diclofenac potassium: NOVARTIS PHARMA S.A.E., Cairo, Egypt). The contents of each tablet were grounded using a mortar and pestle and mixed in an equal amounts by weight (1:1:1) in a mixing pad (100 mg of each). Together with the 50 mg Catafast granules grounded in the same way they were all mixed and dissolved in 100 mL of sterile water to prepare 1 mg/mL of TAPC in a creamy consistency.

3) ***Group C:*** Nano triple antibiotic paste + Nano Catafast (Nano TAPC).

Nano TAPC consists of TAPC: Ciprofloxacin (Ciprofloxacin 500 mg), Metronidazole (Flagyl 500 mg) and Minocycline (Minocin 50 mg), mixed in equal amounts by weight (1:1:1) 100 mg of each, together with the 50 mg Catafast granules. This mixture was transferred into nano structure material using Top-down technique through the combination of various processes including fine grinding, sonication homogenization and ultrafiltration to prevent agglomeration. Ball milling was used to produce nanomaterials by mechanical attrition in which kinetic energy from a grinding medium was transferred to material undergoing reduction.Nanomaterials were put back together with compaction and consolidation in an industrial scale process to form materials with enhanced properties (5).

TEM were performed on JEOL JEM-2100 high resolution transmission electron microscope at an accelerating voltage of 200 kV respectively to test the nano particles' size and shape (fig 1, 2 & 3). The particle size was found to be in the range of 10-40 nms, white in color, powder in form and when tested for solubility it showed dispersion in water.

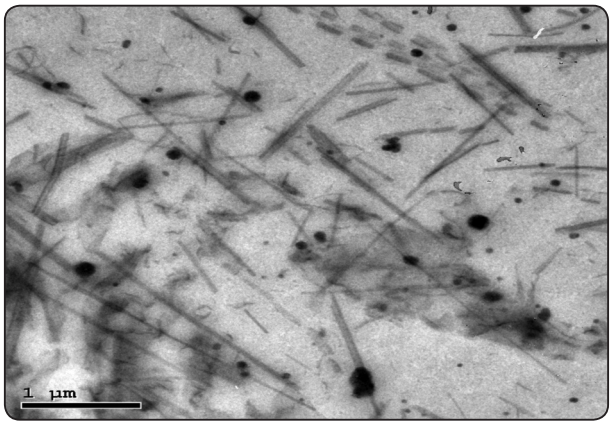

Fig (1): Transmission electron microscope (TEM) images of Nano TAPC with scale bar is 1μm.

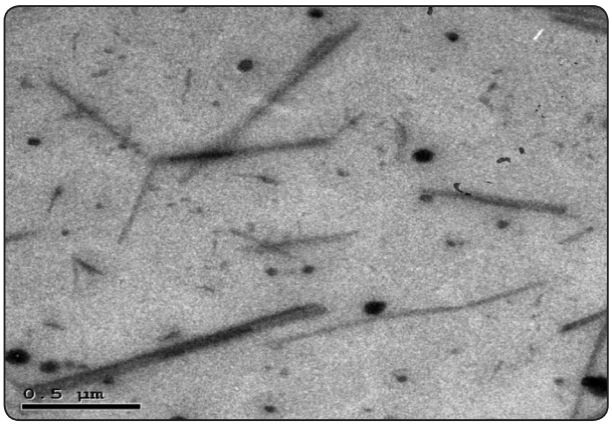

Fig. (2): Transmission electron microscope (TEM) images of Nano TAPC with scale bar is 0.5μm.

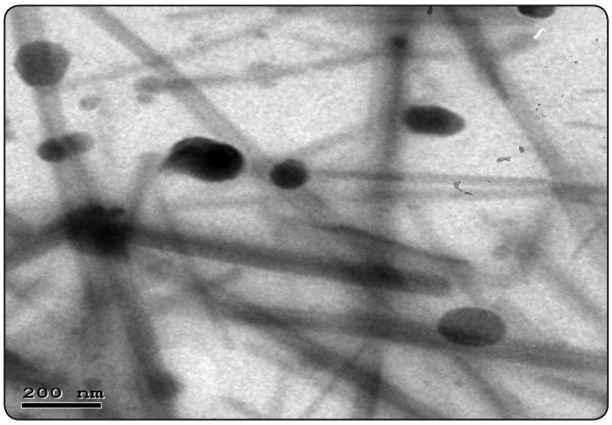

Fig. (3): Transmission electron microscope (TEM) images of Nano TAPC with scale bar is 200 nm.

Scanning Electron Microscope examination with higher magnifications showed that the Nano TAPC particles were highly crystalline with finer crystal size (fig 4&5).

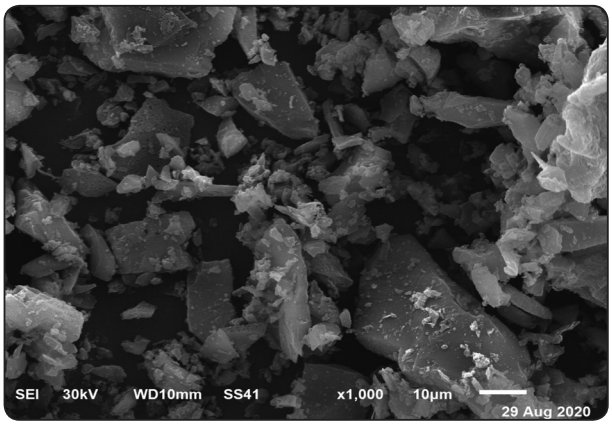

Fig. (4) SEM micrograph (×1000) showing highly crystalline Nano TAPC particles.

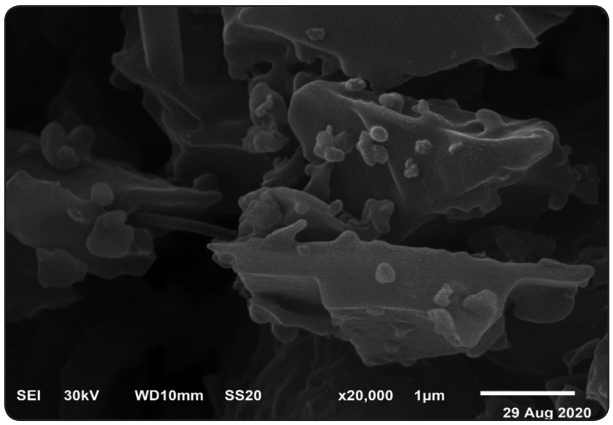

Fig. (5) SEM micrograph (×20,000) showing highly crystalline Nano TAPC particles with fine crystalline size.

The prepared Nano TAPC was be dissolved in 100 mL of sterile water to prepare 1 mg/mL of Nano TAPC

All canals were properly dried then filled with its specified intra-canal medication according to each group, cotton pellet was placed in each pulp chamber and temporary filling was used to close and seal the access cavities. After 72 hours the patients were recalled for the second visit in which the intracanal medication was removed by irrigation using a sterile physiologic saline. The final bacteriological sample (S3) was acquired with the same technique like that done in the first and second bacteriological samples. Laboratory procedures were the same like that done in the first bacteriological sample (2).

RESULTS

Statistical analysis Data presented as mean and standard deviation (SD) when appropriate. Data Explored for Normality using D'Agostino-Pearson test. The significance level was set at $P \leq 0.05$ (α=0.05). Statistical analysis was performed with IBM® SPSS® (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.)

Antibacterial activity

The first bacteriological sample (S1) after access cavity preparation, the mean ± standard deviation

of the bacterial count were 3.84±1.70 for Ca (OH)2 group, 3.85±2.01 for TAPC group and 3.84±1.70 for Nano TAPC. There was no statistically significant difference in bacterial count between the tested groups ($p = 0.982$, $p < 0.05$). (Table 1)

The second bacteriological sample (S2) after cleaning and shaping, the mean ± standard deviation of the bacterial count were 3.63 ± 1.63 for Ca(OH)2 group, 3.36 ± 1.74 for TAPC group and S2 3.36 ± 1.69 for the Nano TAPC. There was no statistically significant difference in bacterial count between the tested groups ($p = 0.467$, $p < 0.05$). (Table 1)

The third bacteriological sample (S3) after intracanal medication application, the mean ± standard deviation of the bacterial count were 2.14±1.36 for Ca(OH)2 group, 1.33±1.39 for TAPC group and 0.5±1.37 for Nano TAPC group. There was a statistically significant difference in bacterial count between the tested groups ($p = 0.01$, $p > 0.05$). (Table 1)

- ***Changes of bacterial count {Log (CFU/ml)} in Ca (OH) 2 group***

There was a decrease in the bacterial count from S1 (3.84±1.70) to S2 (3.63±1.63); the decrease was not statistically significant ($p \geq 0.001$). There was also a decrease in the bacterial count from S2 (3.63±1.63) to S3 (2.14±1.36); the decrease was highly statistically significant ($p \leq 0.001$). (Fig.6&7).

TABLE (1): Mean and Standard deviation (SD) of Bacterial Count (Log (CFU/ml) for different tested groups.

Log (CFU/ml)	Ca(OH)		TAP		Nano TAP		p- value
	Mean	SD	Mean	SD	Mean	SD	
S1	3.84a	1.70	3.85a	2.01	3.84a	1.70	0.982 NS
S2	3.63	1.63	3.36	1.74	3.36	1.69	0.467 NS
S3	2.14b	1.36	1.33b	1.39	0.5b	1.37	0.01*
p-value	≤0.001*		≤0.001*		≤0.001*		

Different letters within each column indicates significant difference.

NS=non-significant, *= Significant ****: Significant at P ≤ 0.05***

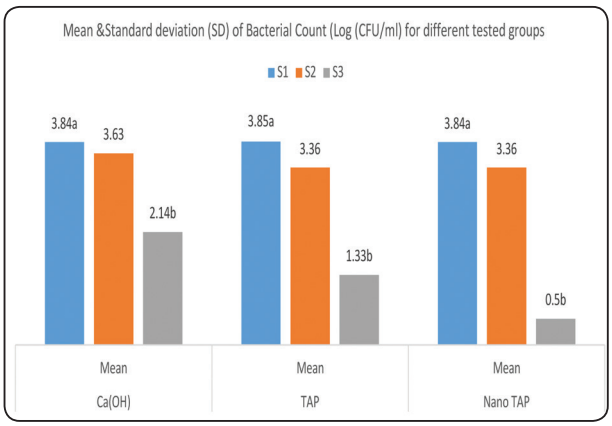

Fig (6). Bar chart showing mean& standard deviation (SD) values of bacterial Count (Log (CFU/ml) for different tested groups.

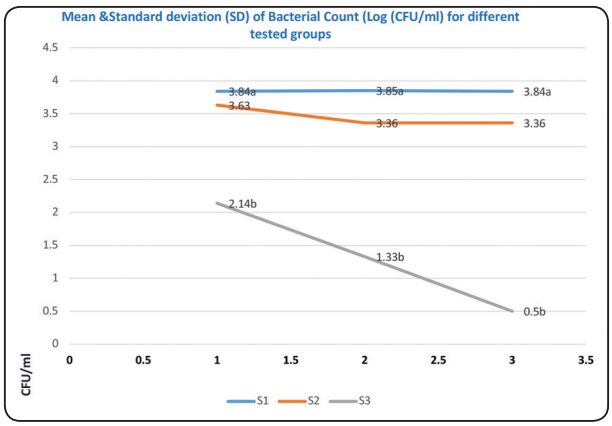

Fig. (7) Mean &Standard deviation (SD) values of bacterial count (Log (CFU/ml) for different tested groups.

- ***Changes of bacterial count {Log (CFU/ml)} in TAPC group:***

There was a decrease in the bacterial count from S1 (3.85 ± 2.01) to S2 (3.36 ± 1.74); the decrease was not statistically significant ($p \geq 0.001$). · There was also a decrease in the bacterial count from S2 (3.36 ± 1.74) to S3 (1.33 ± 1.39); the decrease was highly statistically significant ($p \leq 0.001$). (Fig.6&7).

- ***Changes of bacterial count {Log (CFU/ml)} in Nano TAP group:***

There was a decrease in the bacterial count from S1 (3.84 ± 1.70) to S2 (3.36 ± 1.69); the decrease was not statistically significant ($p \geq 0.001$). There was also a decrease in the bacterial count from S2 (3.36 ± 1.69) to S3 (0.5 ± 1.37); the decrease was highly statistically significant ($p \leq 0.001$). (Fig.6& 7).

DISCUSSION

The Aim of the root canal therapy is to decrease the microbial load within the root canal system to allow healing, this target was usually achieved using mechanical instrumentation of the canals. However, it was proven that this was not enough for total bacterial elimination from the root canal system so different irrigating solutions were used in conjugation with the mechanical instrumentation to achieve this goal. In addition to the use of different formulations of intracanal medicaments applied in between visits in order to disinfect all accessible and inaccessible areas [6].

Intracanal medicaments act by inhibiting proliferation of bacteria after chemo mechanical preparation and reducing ingress of pathogens through temporary restoration leakage in between appointments in multiple visits. The modern concept of intracanal medicaments is that they act as a barrier against the microbial coronal leakage from the gap between filling materials and cavity [7]. These medications should have the ability to work throughout their period of application, they should penetrate deep into the dentinal tubules to eliminate bacteria that may be present and they shouldn't be irritating to the periradicular tissues.

$Ca(OH)_2$ is one of the most commonly used intracanal medicaments in endodontic therapy for decades. It stimulates calcified tissue formation and has a proven high antibacterial effect in 97% of the treated cases after 1 month of the dressing application [8].

TAP is another combination of antibiotics which was introduced especially for the regeneration and revascularization protocol and the treatment of open apex teeth with necrotic pulp. It has been also used in endodontic therapy, its application as an intracanal medicament has reported high antibacterial efficacy[9]. TAP consists of ciprofloxacin, metronidazole, and doxycycline which all act against the pathogens commonly found inside the root canal system including *Enterococcus faecalis*. Root canal infections are considered to be complex because of the encountered diverse flora, this is why combination of antibiotics may be needed to address such complexity.

In asymptomatic uniradicular necrotic teeth the use of both TAPC and $Ca(OH)_2$ as intracanal medications was proved to be efficient in reducing post-operative pain [10].

In the recent years, nanotechnology has become increasingly utilized for medical and dental applications because of their great broad spectrum antibacterial effectiveness. It depends on the production of functional materials and structures in range of 0.1 nm to 100 nm [11].

This study aimed to develop a new nano intracanal medicament; Nano TAPC to evaluate its ability in reducing bacterial count versus regular TAPC and Ca (OH) 2 intra-canal medicament.

Root canals were pre-enlarged to size 25 to allow the paper points to reach the estimated working length without deformation. Root canal samples

were transferred to the laboratory using pre-reduced thioglycolate broth, this medium reduces oxygen, preventing the accumulation of superoxide radicals that would kill anaerobic bacteria. It contains a small quantity of agar that prevents diffusion of oxygen to the medium. This medium is equivalent or superior to pre-reduced anaerobically sterilized transport media because it allows recovery of a greater number of bacteria than other complex media [12].

The high CFU mean counts in the first sample taken (S1) represented prevalence of bacteria in the canals at the beginning of treatment. Chemo mechanical preparation and irrigation with saline resulted in a non-significant decrease in the bacterial concentration in all groups.

Recontamination may occur if the intracanal medicaments remained for more than 3 days so it is recommended to leave them inside the canal only for this duration range [13].

Ca (OH) $_2$ when used as intracanal medicament in the present study achieved the lowest CFU mean counts reduction when being compared to TAPC and Nano TAPC groups, several suggestions have been postulated to explain the limitations of Ca (OH) ^{2}; it has a buffering capacity to dentin, the medicament is neutralized by the bacterial products, the bacteria could be localized in inaccessible areas of the canal that the Ca (OH) 2 cannot reach, some bacterial species have intrinsic resistance to the medicament, insufficient medicament time, some bacterial species have the capability of gene expression alteration that allows them to survive the environmental changes [14]. Furthermore, certain bacteria like Enterococcus faecalis and opportunistic infection causing organisms such as Candida, are found to be highly resistant to Ca (OH) 2 [15]. Locally applied antibiotic pastes were produced and used in the dental market as an alternative to Ca $(OH)_2$ due to their well-known antibacterial and accepted biological properties [16].

After medication with TAPC, the bacterial count dropped observably from 3.36 to 1.33 (Log (CFU/ml) with a statistical significant difference to that of $Ca(OH)_2$ which could be attributed to the combined spectrum of antimicrobial activity and synergetic or additive actions of antibiotics "ciprofloxacin, metronidazole, and minocycline" found in TAP. Ciprofloxacin has a superior effect against both Gram-positive and Gram-negative bacteria by inactivating enzymes and inhibiting cell division [17]. Metronidazole is effective against strict anaerobes prevailing deeply in the dentinal tubules of infected root canals and acts by disrupting bacterial DNA [16]. Minocycline is a broad-spectrum tetracycline antibiotic which acts by inhibiting protein synthesis and inhibiting matrix metalloproteinase enzyme [18]. Combination of these three antibiotics overcomes bacterial resistance and achieves higher antimicrobial action especially when used as topical root canal agents [19]. The absence of inter-appointment flare-up with TAP can be attributed to the antinflamatory property of minocycline [20].

Arruda et al. [21] also found that there was 97% bacterial count reduction from S2 to S3 when antibiotic was used as intracanal medicament while 39% bacterial count reduction was achieved when calcium hydroxide was used. Also Mehta et al [22] found that TAP was the best antibacterial intracanal medicaments followed by CH plus Proton Pump Inhibitors against E. faecalis and C. albicans.

However, TAP can have few drawbacks. It is shown to be the most cytotoxic to human periodontal ligament fibroblasts and cause persistent and exacerbated inflammatory reaction in mouse subcutaneous connective tissue [23]. Also minocycline is said to cause most severe discoloration of the tooth [24] especially in calcifying teeth.

Salem-Milani et al. was the first to comparatively evaluate anti-bacterial efficiency of ibuprofen, diclofenac and Ca $(OH)_2$ using the agar

diffusion test, the authors revealed the anti-bacterial properties of NSAIDs (ibuprofen, diclofenac) against E. faecalis; whereas Ca (OH) 2 failed at the same [25]. The exact mechanism of antibacterial activity of diclofenac and ibuprofen remains unclarified. Studies have postulated the following mechanism's of action: inhibition of bacterial DNA synthesis, impairment of membrane activity, anti-plasmid activity, alteration in genes encoding transport/binding proteins, DNA synthesis and cell envelope as well as down-regulation of efflux pumps, reduced quorum sensing-controlled motility leading to reduced biofilm [26].

Diclofenac potassium anti-inflammatory drug was proved to be efficient in reducing post-operative pain when it was added to triple antibiotic paste as intracanal medication, its antinflamatory effect was comparable to that of calcium hydroxide when used in cases of asymptomatic uniradicular necrotic teeth [10].

The Nano TAPC group showed the lowest bacterial count (0.5 ± 1.37) with a statistically significant difference than the other two groups. The characteristics of nanoparticles including the particle size, concentration, contact time and surface charge, influence their antimicrobial action against bacterial cells and mature biofilm [27].

The higher antibacterial activity of the nano form of the TAPC intracanal medicament could be attributed to fine particle size (10-40 nms), highly crystalline particles' form which gave the ability of the nanoparticles to bind to the negatively charged part of the bacterial cell membrane, disturbing its functions such as permeability and respiration, causing leaking of the cytoplasmic content and eventually rupture of the bacterial cell as it was reported by Ibrahim et al. [28]. The nanoparticles infiltrate inside the cytoplasmic content and interact with sulphur and phosphorus containing proteins such as DNA and RNA [29] which leads to formation of Reactive Oxygen Species ROS due to oxidative DNA damage [30]. Oxidative stress occurs when generation of ROS exceeds the capacity of the cellular antioxidant defense system. Depletion of glutathione and protein bound sulf hydryl groups and changes in the activity of various antioxidant enzymes indicative of lipid peroxidation have been implicated in oxidative damage [31].

CONCLUSION

The use of the Nano TAPC as intracanal medicament showed the highest bacterial reduction count when compared to TAP and Ca (OH) $_2$.

Conflict of interests

The author declares that there is no conflict of interest related to this research and there is no financial involvement with any commercial organization in the subject or materials discussed in this manuscript.

Highlights key points

- Root canal system disinfection is a difficult task that depends on both proper chemo-mechanical preparation as well as efficient intracanal medicaments.
- Nano TAPC have significantly effective antimicrobial activity when used as intracanal medicament compared to TAPC and Ca (OH) 2.

REFERENCES

1. Gilad JZ, Teles R, Goodson M, White RR, Stashenko P. Development of a clindamycin-impregnated fiber as an intracanal medication in endodontic therapy. J Endod 1999; 25: 722-7.
2. Windley W 3rd, Teixeira F, Levin L, Sigurdsson A, Trope M. Disinfection of immature teeth with a triple antibiotic paste. J Endod. 2005; 31: 439–43.
3. Markan S, Lehl G, Kapoor S. Recent Advances of Nanotechnology in Endodontics , Conservative and Preventive Dentistry-A Review. 2017;2(10):1–4.
4. Manzur A, González AM, Pozos A, Silva-Herzog D, Friedman S. Bacterial Quantification in Teeth with Apical Periodontitis Related to Instrumentation and Different

Intracanal Medications: A Randomized Clinical Trial. J Endod 2007; 33: 114–8.

5. V.M.Arole. Fabrication of nanomaterials by Top-Down and Bottom-Up approaches-An overview. Journal of Advances in Applied Sciences and Technologies 2014; 1: 89-93.

6. Afkhami F, Elahy S, Mahmoudi-Nahavandi A. Spectrophotometric analysis of crown discoloration following the use of silver nanoparticles combined with calcium hydroxide as intracanal medicament. J Clin Exp Dent 2017;9:842–7.

7. Kumar A, Tamanna S, Iftekhar H. Intracanal medicaments – Their use in modern endodontics : A narrative review. J Oral Res Rev 2019;11:94–9.

8. Byström A, Claesson R, Sundqvist G. The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol and Calcium hydroxide in the treatment of infected root canals. Endod Dent Traumatol 1985;1(5):170-5.

9. Parhizkar A, Nojehdehian H, Asgary S. Triple antibiotic paste : momentous roles and applications in endodontics : a review. Restor Dent Endod 2018;43:1–16.

10. Omaia M, Negm M, Nashaat Y, Nabil N and Othman A. The effect of triple antibiotic paste as an intracanal medication with an anti-inflammatory drug on post-operative pain of asymptomatic uniradicular necrotic teeth: a double blind randomized clinical trial, F1000Research 2019; 8:1687-97.

11. Markan S, Lehl G, Kapoor S. Recent Advances of Nanotechnology in Endodontics , Conservative and Preventive Dentistry-A Review. 2017;2(10):1–4.

12. Zamany A, Safavi K, Spangberg LS. The effect of chlorhexidine as an endodontic disinfectant. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003; 96: 578–81. 18.

13. Akcay M, Arslan H, Topcuoglu HS, Tuncay O. Effect of calcium hydroxide and double and triple antibiotic pastes on the bond strength of epoxy resin-based sealer to root canal dentin. J Endod. 2014; 40: 1663–7.

14. Nerwich A, Figdor D, Messer HH. PH changes in root dentin over a 4-week period following root canal dressing with calcium hydroxide. J Endod 1993; 19: 302– 6.

15. Waltimo TM, Sirén EK, Orstavik D, Haapasalo MO. Susceptibility of oral Candida species to calcium hydroxide in vitro. Int Endod J. 1999; 32: 94–8.

16. Taneja S, Kumari M. Use of triple antibiotic paste in the treatment of large periradicular lesions. J Investig Clin Dent. 2012; 3: 72–6.

17. Thibodeau B, Teixeira F, Yamauchi M, Caplan DJ, Trope M. Pulp revascularization of immature dog teeth with apical periodontitis. J Endod. 2007; 33: 680–9.

18. Brunton LL, Lazo JS, Parker KL. 11th ed. New York: McGraw Hill companies; 2006. Goodman and Gilman's the pharmacological basis of therapeutics; 1111–26. (1049-72). 1182-7.

19. Alam T, Nakazawa F, Nakajo K, Uematsu H, Hoshino E. Susceptibility of Enterococcus faecalis to a combination of antibacterial drugs (3 mix) in vitro. J Oral Biosci. 2005; 47: 315–20.

20. Dunston CR, Griffiths HR, Lambert PA, Staddon S, Vernallis AB. Proteomic analysis of the anti-inflammatory action of minocycline. Proteomics. 2011; 11: 42–51.

21. Arruda MEF, Neves MAS, Diogenes A, et al. Infection Control in Teeth with Apical Periodontitis Using a Triple Antibiotic Solution or Calcium Hydroxide with Chlorhexidine: A Randomized Clinical Trial. J Endod 2018; 22. pii: S0099-2399(18)30462-X.

22. Mehta S, Verma P, Tikku AP, et al. Comparative evaluation of antimicrobial efficacy of triple antibiotic paste, calcium hydroxide, and a proton pump inhibitor against resistant root canal pathogens. Eur J Dent 2017; 11: 53–7.

23. Pereira MS, Rossi MA, Cardoso CR, et al. Cellular and molecular tissue response to triple antibiotic intracanal dressing. J Endod 2014; 40: 499-504.

24. Lenherr P, Allgayer N, Weiger R. Tooth discoloration induced by endodontic materials: A laboratory study. Int Endod J. 2012; 45: 942–9.

25. Salem-Milani A, Balaei-Gajan E, Rahimi S, et al. Antibacterial effect of diclofenac sodium on Enterococcus faecalis. J Dent (Tehran) 2013; 10:16–22.

26. Mazumdar K, Dastidar SG, Park JH, Dutta NK. The anti-inflammatory non-antibiotic helper compound diclofenac: An antibacterial drug target. Eur J Clin Microbiol Infect Dis. 2009; 28: 881–91.

27. Abbaszadegan A, Nabavizadeh M, Gholami A, Aleyasin ZS, Dorostkar S, Saliminasab M, et al. Positively charged imidazolium-based ionic liquid-protected silver nanoparticles: a promising disinfectant in root canal treatment. International Endodontic Journal. 2015; 48: 790–800.

28. Ibrahim AIO, Moodley DS, Petrik L, Patel N. Use of antibacterial nanoparticles in Endodontics. South African Dent J 2017;72:105–12.

29. Dianat O, Saedi S, Kazem M. Antimicrobial Activity of Nanoparticle Calcium Hydroxide against Enterococcus Faecalis: An In Vitro Study. Iran Endod J 2015;10(1) 39-43.

30. Bresan E, Vindignii V, Ferronii L, Caiirns W, Gardinn C, Rigoo C, Zavann B, Stocchero M. Silver Nanoparticles and Mitochondrial Interaction. Int J Dentistry. 2013;2013:1–8.

31. Danielle Mc, Shan Paresh C, RayHongtao Yu . Molecular toxicity mechanism of nanosilver. Journal of Food and Drug Analysis Volume 22, Issue 1, March 2014, Pages 116-127.