EGYPTIAN DENTAL JOURNAL
www.eda-egypt.org
VOL. 71, 931:938, JANUARY, 2025
PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360
CONSERVATIVE DENTISTRY AND ENDODONTICS
Submit Date : 17-11-2024 • Accept Date : 11-12-2024 • Available online: 10-01-2025 • DOI : 10.21608/edj.2024.333225.3255

EVALUATION OF THE ANTIBACTERIAL EFFICACY OF DICLOFENAC AS INTRACANAL MEDICAMENT VERSUS TRIPLE ANTIBIOTIC PASTE AGAINST ENTEROCOCCUS FAECALIS IN SINGLE-ROOTED TEETH. (A COMPARATIVE IN-VITRO STUDY)

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ABSTRACT

Aim: To evaluate the antibacterial efficacy of diclofenac potassium as intracanal medicament versus triple antibiotic paste against *Enterococcus faecalis* biofilm inoculated in single root canals by bacterial count assessment.

Methodology: Forty-two extracted human mandibular premolars with single canals were instrumented manually up to size #25 then sterilized in an autoclave before inoculation with *E. faecalis and* then kept at 37°C. The 1st sample (S1) was taken after 14 days. The root canals were prepared up to #40/04 (MG3 Gold), then the 2nd sample (S2) was taken. The specimens were randomly distributed among 3 groups (n=14), the 1st group: (triple antibiotic paste TAP), 2nd group; (diclofenac potassium paste DP), and the 3rd group (no medicament NC). After sealing the specimens, they were incubated for 7 days then the 3rd sample (S3) was taken after medicament removal. Visual counting of bacterial colonies on agar plates using the measuring unit (CFU/ml) was used to assess the bacterial count.

Results: The differences between the (TAP), (DP), and (NC) groups were statistically significant ($p<0.001$). Regarding S3 samples, a statistically significant difference was found between (NC) group and each of (TAP and DP) groups where ($p<0.001$) while no significant difference was found between (TAP) and (DP) where ($p=0.098$).

Conclusions: Diclofenac potassium shows antibacterial action comparable to triple antibiotic paste against *E. faecalis*.

KEYWORDS: *E. faecalis*, triple antibiotic paste, diclofenac potassium, antibacterial, colony-forming units.

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INTRODUCTION

Prevention or management of apical periodontitis is considered the main purpose of root canal therapy. One of the principal causes of endodontic failure is persistent infection (post-treatment disease). *Enterococcus faecalis* was considered the most prevalent microorganism isolated from chronic apical periodontitis and periapical lesions associated with failed endodontic treatment with prevalence values reaching up to 90% of the cases [(1)].

Root canal debridement and disinfection have a magnificent role in elimination and prevention of apical periodontitis through the predictable eradication of microorganisms and their byproducts. Using intracanal medicaments after thorough debridement can improve the disinfection of the root canals and increase the success of root canal therapy. Since root canal infections are polymicrobial, it is common for some bacteria to develop resistance if a single antibiotic is used [(2)].

Hence, Triple antibiotic paste (TAP) was introduced by (**Hoshino *et al.***) [(3)] which was composed of metronidazole, ciprofloxacin, and minocycline. The mixture showed antibacterial effectiveness in infected root canals and was potent in eradicating bacteria in necrotic and resistant cases. It also creates a suitable environment for pulp revascularization/regeneration [(4)]. The major drawback of using triple antibiotic pastes was tooth discoloration related to minocycline [(5)].

Non-steroidal anti-inflammatory drugs (NSAIDs) were found to have an antibacterial activity related to ibuprofen for the first time in 1995 [(6)]. Furthermore, diclofenac sodium was found effective against drug-resistant and drug-sensitive clinical isolates of *Staph. aureus, L. monocytogenes, E. coli,* and *Mycobacterium* species [(7,8)]

Diclofenac sodium and ibuprofen demonstrated antibacterial activity when investigated in many in-vitro studies against *E. faecalis* when compared with calcium hydroxide or triple antibiotic paste [(9-11)] Also, diclofenac sodium increased the anti-biofilm effect of calcium hydroxide [(12)].

The antibacterial activity of diclofenac is believed to be through inhibiting synthesis of bacterial DNA. Since both formulations of diclofenac (potassium and sodium) have shown similar antibacterial effects; the aim of the present study was to evaluate the antimicrobial efficacy of diclofenac potassium paste compared to triple antibiotic paste against *Enterococcus faecalis* biofilm developed in teeth with single root canals. The null hypothesis was that there is no difference between the antimicrobial efficacy of diclofenac potassium and triple antibiotic paste in reduction of *E. faecalis* count in single rooted teeth.

MATERIALS AND METHODS

All procedures were approved by the research ethical committee, Faculty of Dentistry, Cairo University (Approval number: 30/5/2023) concerning the scientific content and compliance with applicable rehearse and human subjects and regulations. After obtaining the data and finishing the experiment, the Microbiology Department at Cairo University sanitized and burnt all apparatus and tooth samples in a separate incinerator.

Sample Size Calculation:

The sample size was determined using PS software version 3.1.2. In the study by (Chockattu et al., 2018), the mean bacterial load after 7 days of the diclofenac group was found to be (0.42 ± 1.84). Assuming a minimum significant difference of 2 colony-forming unit (CFU) counts, a type I error of 0.05, and a study power of 0.8, the sample size needed to recognize a significant difference between the experimental groups was found to be 42 samples (14 samples per group).

Sample Preparation:

Forty-two human permanent single-rooted mandibular premolars extracted for orthodontic, periodontal, or prosthodontic reasons were collected. Soft tissue remnants were removed by soaking teeth in 5.25% NaOCl solution for 15 mins, then hard deposits were removed using an ultrasonic scaler. Using a low-speed diamond disc, the crowns of each tooth were decapitated and adjustment was made at the apical foramen to obtain a uniform root length of 15 mm. Vertucci type I root canal system was confirmed by radiographic assessment from both buccolingual and mesiodistal dimensions. Canal patency was established by extending #15 K-file beyond the apex, then canals were instrumented up to #25 K-file (MANI, Co., Tochigi-Ken, Japan) after working length determination. After that, root apices were sealed by resin composite (3M ESPE Composite Z250XT, 3M ESPE Dental products, U.S.A), two layers of colorless nail polish were applied to the external surface of all teeth to avoid liquid seepage (Yolo, Yolo Cosmetics, France). A 30-minute sterilization at 121°C was done for all samples in an autoclave, to guarantee the negative culture and sample sterilization, one tooth was chosen at random and sampled.

Sample Contamination and Root Canal Preparation:

A pure standard strain of *Enterococcus faecalis* (ATCC 29212) was revived on bile esculin agar at 37°C for 24 h. The bacterial suspension was prepared in sterile brain heart infusion broth (BHI) and turbidity was adjusted to 0.5 McFarland, equivalent to ($1.5x10^8$) CFU/mL.

Samples were placed in sterile Eppendorf tubes containing brain heart infusion (BHI) broth, 50 μL of bacterial suspension was placed inside each root canal using a micropipette. The samples were incubated at 37°C for 14 days in an incubator (Fisher Isotep* Incubator. WTC Binder, Tuttlingen, Germany). During this period, refreshment of the BHI broth was done every 3 days.

After incubation, teeth were rinsed thoroughly with 5 ml of saline solution to eliminate excess broth then the 1st sample (S1) was collected with three successive paper points size (#25/02). For Chemo-mechanical preparation, all teeth were prepared with MG3 Gold rotary NiTi instruments (MG3 Gold, Perfect Medical Instruments Co., Ltd., Shenzhen, China.) up to (#40/04). The canals received 2 ml of 2.5% sodium hypochlorite solution (Clorox, Household Cleaning Products Company, 10th of Ramadan, Egypt) between each instrument, using a double side-vented needle inserted 1 mm shorter than the working length.

Each canal received 3 ml of NaOCl as a final flush, followed by 3 ml of sterile saline solution then 3 ml of 17% EDTA solution left for 1 min., then a final rinse with 3 ml saline was done. Root canals were properly dried, and the second bacteriological samples (S2) were taken immediately after the irrigation protocol.

Intracanal medicaments preparation and placement:

Diclofenac Potassium paste (I):

Diclofenac potassium powder (Catafast, NOVARTIS PHARMA S.A.E, Cairo, C.C.R) was obtained from a sachet. Each sachet contains 50 mg of diclofenac potassium in a powder weight of 2 g.

Triple Antibiotic Paste (C):

To prepare TAP, a 500 mg tablet of metronidazole (Flagyl, Sanofi, Egypt) and a 500 mg tablet of ciprofloxacin (Ciprofloxacin, ORGANOPHARMA, Egypt) were crushed into powder then 5 capsules of doxycycline (Vibramycin, Pfizer, Viatris Egypt) (100 mg per capsule) were added and well mixed.

Both medicaments were mixed with distilled water at a concentration (1 mg/ml) on a sterile glass

slab to form a paste, then they were inserted inside the root canals with an MTA carrier and packed with an endodontic plugger size (40*80) (Fanta, Fanta Dental, China). Each tooth was covered by sterile tin foil and stored in an Eppendorf tube and incubated at 37°C for 7 days.

No Medicament group:

After S2 sampling, teeth orifices were sealed with sterile gauze then covered with sterile tin foil. Teeth were placed inside Eppendorf tubes and incubated for 7 days at 37°C.

Medicaments Removal:

In medicated groups, root canals were rinsed by 5 ml irrigation with sterile saline by a side vented needle combined with manual agitation with (#25/02 H-file), then each canal was dried and sampled with 3 paper points for post-medication samples (S3). In the non-medicated group, root canals were rinsed with 5 ml of saline solution, then sampled as the previous groups.

Outcome Assessment:

Visual counting of bacterial colonies on agar plates using the measuring unit (CFU/ml) was used to assess the bacterial count. The paper points collected from each sample (S1, S2, and S3) were placed inside an Eppendorf tube containing saline solution. The Eppendorf tubes were vortexed for the dispersion of the samples for 1 min. A volume of 50 μL of the dispersed solution was placed over BHI agar plates and cultured aseptically and incubated at 37°C for 24 hrs. Only in (S1) samples, a serial fold of two dilutions (1/10, 1/100) was done to determine *E. faecalis* count in each root canal.

Statistical Analysis:

Data were summarized using mean and standard deviation and tested for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. One-way ANOVA followed by Tukey post-hoc test was used to compare more than two groups in non-related samples. To compare between more than two groups in related samples, repeated measure ANOVA was used. Paired sample t-test was utilized for comparison between two groups in related samples.

RESULTS

I) Relation between S1, S2 and S3:

In all groups, there was a statistically significant reduction in bacterial count between (S1) and each of (S2) and (S3) where ($p<0.001$). Also, a statistically significant difference was detected between (S2) and (S3) where ($p<0.001$) in all groups.

The greatest mean value was detected in (S1) samples followed by (S2) samples, while the least mean value was found in (S3) samples in TAP and diclofenac (DP) groups.

Only in the non-medicated group (negative control or NC), the highest mean value was found in (S1) samples followed by (S3) samples, while the lowest mean value was found in (S2) samples.

II) Relation between Groups:

Sample 2 (S2):

No statistically significant difference was found between the three tested groups where ($p=0.087$). The mean and standard deviation values for TAP, diclofenac and negative control groups are represented in (Table 1) and (Fig.1).

Sample 3 (S3):

NC group showed a statistically significant difference than the two other groups (TAP and DP) where ($p<0.001$). The negative control group had a higher mean value than TAP and DC groups. No statistically significant difference was detected between (TAP) and (DP) where ($p=0.098$). The mean and standard deviation values for TAP, diclofenac and negative control groups are represented in (table 1) and (fig.1).

TABLE (1) The mean, and standard deviation (SD) values of bacterial count of different groups in each sample.

	Bacterial Count (CFU/ml)						
	S1		S2		S3		p-value
	Mean	SD	Mean	SD	Mean	SD	
Triple antibiotic paste (TAP)	285.71 ^{aA}	42.08	3.27 ^{aB}	0.50	0.93 ^{bC}	0.29	**<0.001***
Diclofenac Potassium (DP)	372.86 ^{aA}	49.06	1.79 ^{aB}	0.35	0.79 ^{bC}	0.26	**<0.001***
Negative control (NC)	412.86 ^{aA}	32.92	2.29 ^{aC}	0.45	35.79 ^{aB}	8.46	**<0.001***
p-value	**0.103ns**		**0.087ns**		**<0.001***		

Means with different lower-case in the same column indicate statistically significance difference, means with different upper-case letters in the same row indicate statistically significance difference. *; significant (p<0.05) ns; non-significant (p>0.05)

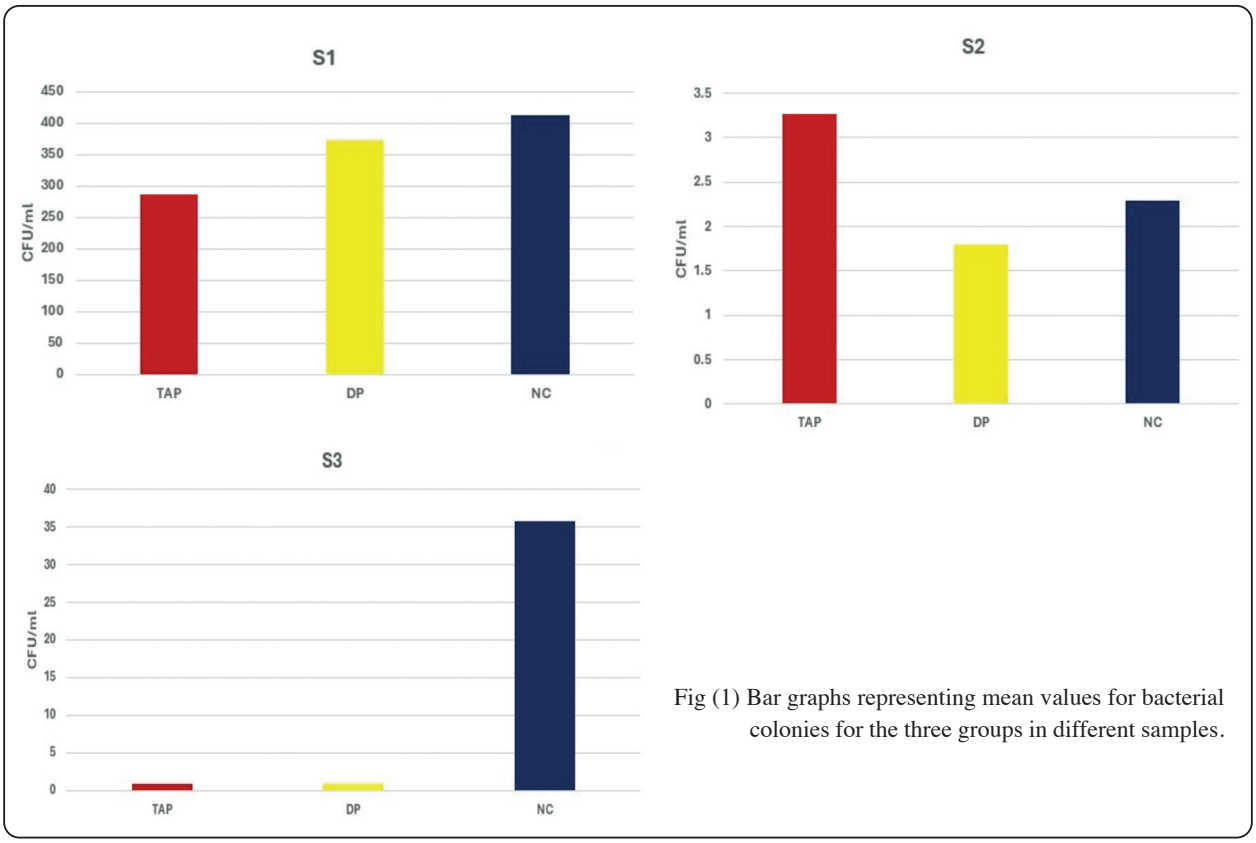

Fig (1) Bar graphs representing mean values for bacterial colonies for the three groups in different samples.

DISCUSSION

The current study aimed to evaluate the antimicrobial efficacy of two different canal medicaments against a standardized strain of *E. faecalis* (ATCC 29212). It was found that diclofenac potassium reduced the intracanal bacterial count similar to triple antibiotic paste, so the null hypothesis failed to be rejected.

The experimental model used for this study was chosen to mimic clinical conditions. Single-rooted teeth with single canals were used for the study to exclude anatomical variations. Teeth apices were sealed and sterilized then contaminated with E. faecalis [13]. The first bacteriological sample (S1) was taken before chemo-mechanical preparation) to verify the viability of bacteria [14,15].

Root canals were prepared with MG3 gold heat-treated files. This file sequence has variability in cross-section designs providing high cutting efficiency, enhanced flexibility, and cyclic fatigue resistance properties.

Triple antibiotic paste was chosen for the positive control group intracanal medication. (TAP) was introduced especially for regenerative endodontic procedures and the treatment of open apex teeth with necrotic pulp. Besides, (TAP) showed the potential for healing large periapical, cystic, or cyst-like lesions and resistant root canal infections [16].

Particularly, (TAP) showed a distinctive antibacterial effect against *E. faecalis* compared to calcium hydroxide [17,18] and chlorohexidine gel [19,20].

Doxycycline was used instead of minocycline as it was unavailable in the market. Moreover, it was reported that minocycline was associated with the highest percentage of coronal discoloration among other substitutes [5,21]. (TAP) powder was mixed with distilled water (w/v ratio of 1:1) to obtain a homogenous paste.

Diclofenac potassium was chosen as the tested intracanal medicament in our research. Previously, diclofenac sodium was commonly tested for its antimicrobial properties [9,11,12,22]. **Abdalkrim et al., 2023** [23] compared the antibacterial activity of diclofenac potassium and sodium against Staphylococcus aureus and MRSA, their results showed a non-significant difference in antibacterial activity for both drugs. This result agreed to our pilot study results as well.

Salem-Milani *et al*., 2013 [22] determined the minimum inhibitory concentration of diclofenac sodium is 50 μg/ml and above, however, this concentration is clinically inapplicable as mentioned by **(Chockattu *et al*., 2018)** [11]. Therefore, diclofenac potassium powder was mixed with distilled water in (w/v ratio 1:1) similar to TAP preparation.

Regarding intra-group comparisons, our results have shown a statistically significant difference between S1 and S2 samples in all groups, this illustrates the substantial role of chemo-mechanical procedures in the reduction of intracanal bacterial populations and biofilm disruption. Also, a statistically significant difference was found between S2 and S3 values in (TAP and DP) groups where mean values for S2 were higher than those for S3. Our result agreed with **(Windley *et al*., 2005)** [13] and **(Abd El-Majeed *et al*., 2020)** [24].

Conversely, the mean values of S3 were higher than those of S2 in the non-medicated group. This outcome leads to a logical conclusion supported by literature considering multiple visit endodontic treatment stating that when no inter-appointment intracanal dressing is applied, there is a certain route for re-infection of the root canal space [25,26].

Concerning inter-group comparisons, no statistically significant difference was found between (TAP) and (DP) groups for (S3) samples. This finding about diclofenac potassium agreed with the previous results of **(Tilokani *et al*., 2023)** [27] when they found no significant difference in bacterial reduction of diclofenac sodium and triple antibiotic paste. Moreover, **(Chockattu *et al*., 2018)** [11] compared ibuprofen and diclofenac with calcium hydroxide, their conclusion was diclofenac could be

an alternative medicament for calcium hydroxide. **(Abu naeem *et al*., 2022)** [14] and **(Rezk *et al*., 2021)** [10] found similar results to ours when comparing triple antibiotic paste with ibuprofen as well.

According to our results, diclofenac could be a valid alternative to the standard intracanal medicaments such as calcium hydroxide and triple antibiotic paste. NSAIDs are showing very promising potential in root canal disinfection, in addition to their anti-inflammatory properties.

CONCLUSIONS

Within the constraints of this in-vitro study, diclofenac potassium shows antibacterial action comparable to triple antibiotic paste. Further research is needed to validate using diclofenac potassium as an alternative intracanal medicament in controlling resistant intracanal infections and symptomatic periapical lesions.

Conflict of interest:

No conflict of interest is to be declared.

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