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Original Article

Dentistry Section

Evaluation of Antimicrobial Efficacy of Modified Novel Double Antimicrobial Preparations Against *E.Faecalis* and *C. Albicans*: An In-vitro Study

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ABSTRACT

Introduction: The aim of endodontic treatment is the reduction of microbial load inside the root canal, for which Calcium hydroxide {$Ca(OH)_2$} is the most commonly used medicament, which is ineffective against resistant microbes such as *E.faecalis* and *C.albicans*. Hence, this study was designed to develop an effective medicament which includes a combination of drugs such as diclofenac, ciprofloxacin, fluconazole, with Ethyl Cellulose (EC).

Aim: To evaluate the antimicrobial efficacy of different combinations of Diclofenac, Ciprofloxacin and Fluconazole (DCF) with EC against *E.faecalis* and *C.albicans*.

Materials and Methods: This in-vitro study, conducted at the Department of Conservative Dentistry and Endodontics in RVS Dental College and Hospital, Coimbatore, Tamil Nadu, India, from September 2024 to January 2025. This included quality control strains of *C.albicans* and *E.faecalis*, which were cultured in Tryptic soy broth supplemented with 5% defibrinated sheep blood under anaerobic conditions at 37°C for 45 hours. The two groups (*E.faecalis* group and *C.albicans* group) with test materials, which were further divided into three groups: Group I-Calcium hydroxide, Group II-Saline, and Group III-Test drugs. Test drugs were prepared by making different combinations of drugs {Active Pharmaceutical Ingredient (API) powders} of the test drugs, weighed and mixed in 1:10, 2:10, 3:10 ratios with Ethylcellulose polymer (Test drugs-Diclofenac, Ciprofloxacin and Fluconazole). A sample of three different concentrations was examined across all combinations of drug preparations. The antimicrobial activity was assessed using the disk diffusion method. Microbial lawns were prepared on Mueller-Hinton plates with inoculum standardised to 1.5×10^8 CFU/mL. A disk (6 mm diameter) was punched aseptically and filled with 20 µL of test materials. Wassermann filter paper discs were incubated at 37°C for 24 hours, and zones of inhibition were measured using a HiMedia antibiotic scale. Data were statistically analysed using One-way Analysis of Variance (ANOVA) in the Statistical Package for Social Sciences (SPSS) software 24.0.

Results: ANOVA statistics revealed that DCF (3:10) consistently demonstrated the highest antimicrobial efficacy against both *E.faecalis* and *C.albicans*, with a p-value of <0.001. These findings suggest that novel antibiotic combinations, especially those incorporating Diclofenac, may serve as effective therapeutic agents in combating resistant pathogens.

Conclusion: Within the limitations of the study, it can be concluded that DCF (3:10) consistently demonstrated the highest antimicrobial efficacy against both *E.faecalis* and *C.albicans*; therefore, it can be considered a potential intracanal medicament against these pathogens.

Keywords: Anti-bacterial agents, Anti-infective agents, Endodontic treatment

INTRODUCTION

Microbial infections affecting the dental pulp and root canals may result in an inflammatory condition in the periradicular tissues, referred to as apical periodontitis. Various bacterial species contribute to these conditions, including those from the groups Fungicides, Actinomycetes, Fusobacteria, Spirochaetes, and Bacteroides (Siqueira JF and Rôças IN 2022). Techniques for culture and identification have indicated that fungicides and bacteroides are linked to the onset of irreversible pulpitis and periapical periodontitis [1].

Fungi are often implicated in cases of infected root canals, having been isolated in approximately 3-18% of such instances, with Candida species being the most prevalent. A systematic review and meta-analysis indicated that *Candida albicans* is the most frequently isolated fungus from infected root canals, followed by other species such as Candida tropicalis, Candida kefyr, Candida parapsilosis, Candida glabrata, Candida krusei, Candida dubliniensis, Candida guilliermondii, and Candida etchellsii [2].

C.albicans adheres to both biotic and abiotic surfaces, including dental prosthetics and tooth dentin. It exhibits a preference for dentin, colonising the walls of root canals and infiltrating the dentinal tubules to establish biofilms. The spherical *C.albicans* cells attach to dentin surfaces within 60 to 90 minutes, subsequently forming a foundational layer of biofilm that matures over 24 hours. These mature biofilms consist of multiple layers of approximately 20 polymorphic cells, which include hyphal, pseudohyphal, and yeast forms, all embedded in extracellular matrices that create robust and physiochemically resilient structures [2].

Following maturation, the round yeast cells can disperse to infect other sites. *C.albicans* within these biofilms exhibits a 10 to 100-fold increase in resistance to host immune responses and antifungal treatments, as the growth and metabolism of the cells are shielded by the extracellular matrices, which consist of Extracellular Polymeric Substances (EPS) and protective factors [3]. Consequently, *C.albicans* in biofilms is significantly more challenging to eliminate than planktonic cells and is frequently associated with persistent or refractory endodontic infections that do not respond to conventional root canal therapies [3].

Enterococcus faecalis is a gram positive facultative anaerobe belonging to the Firmicutes phylum. This bacterium exhibits remarkable resilience and is commonly associated with infections of the dental pulp, resulting in persistent root canal infections [4].

The specific effects of *Enterococcus faecalis* on pulp and periapical tissues remain poorly understood. It produces various byproducts, including lysase, gelatinase, hyaluronidase, and cytolysin, which can lead to tissue damage or alter the immune responses of pulp cells, potentially exacerbating tissue injury. Additionally, this bacterium has been shown to inhibit osteoblast differentiation and to enhance the expression of osteogenic genes in human Dental Pulp Stem Cells (hDPSCs), which may influence the healing processes of pulp and periapical lesions [5].

Calcium hydroxide {$Ca(OH)_2$} is the most commonly used intracanal medicament in endodontics. However, its efficacy against resistant microorganisms is limited [6]. Diclofenac is identified as 2-(2,6-dichloranilino) phenylacetic acid. It is available in both sodium and potassium salt forms, both of which exhibit high solubility in solvents like methanol and Dimethyl Sulfoxide (DMSO). Notably, the majority of research examining the effects of diclofenac on bacteria has utilised its sodium variant. This Non Steroidal Anti-inflammatory Drug (NSAID) has demonstrated antimicrobial properties in-vitro against various bacterial pathogens [7]. Ciprofloxacin is a synthetic fluoroquinolone antibiotic known for its broad-spectrum antibacterial properties. Antimicrobial and analgesic medications are often used in combination to alleviate pain associated with various infections [8]. Fluconazole targets the cytochrome P450 enzyme lanosterol demethylase (14-demethylase), which plays a crucial role in the biosynthesis of ergosterol in fungi, thereby disrupting cell membrane formation. It is primarily available in enteral and intravenous forms, but can also be found as a mouthrinse or suspension for treating localised infections. The effectiveness of systemic fluconazole in both preventing and treating oropharyngeal and oesophageal candidiasis is linked to the significant concentrations reached in salivary secretions after oral intake [9].

The EC is a significant derivative of natural cellulose. EC is a food additive that has received approval from the Joint Food and Agriculture Organisation of the United Nations/World Health Organisation (FAO/WHO). It forms oleo gel; these gels have a semi-solid structure, which is a thickener used in our study to make drug preparation viscous [10].

To address this challenge, this study aimed to develop a novel medicament combining diclofenac, ciprofloxacin, and fluconazole. This study was formulated to evaluate its antimicrobial potential against resistant organisms.

The present study aimed to evaluate the antimicrobial efficacy of different combinations of Diclofenac, Ciprofloxacin, and Fluconazole with EC against *Enterococcus faecalis* and *Candida albicans*, compared to calcium hydroxide. To date, no studies have formulated this particular combination or evaluated its antimicrobial efficacy; therefore, this study is being conducted.

The null hypothesis of this study posits that there is no difference in the antimicrobial efficacy of the various combinations of Diclofenac, Ciprofloxacin, and Fluconazole with EC against *E.faecalis* and *C.albicans* when compared to calcium hydroxide. In contrast, the alternative hypothesis suggests that there is greater antimicrobial efficacy in the combinations of Diclofenac, Ciprofloxacin, and Fluconazole with EC against *E.faecalis* and *C.albicans* compared to calcium hydroxide.

The primary objective of this study was to evaluate the antimicrobial efficacy of these different combinations using the zone of inhibition at three different drug combinations against *E.faecalis* and *C.albicans* compared to calcium hydroxide.

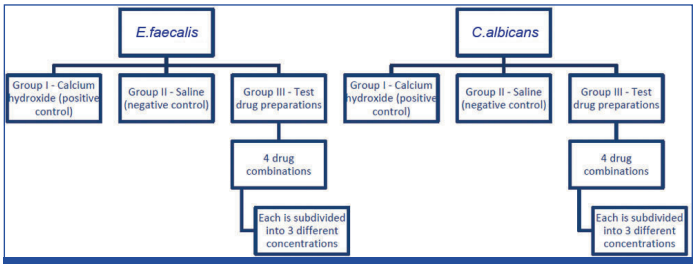
MATERIALS AND METHODS

This in-vitro study, conducted at the Department of Conservative Dentistry and Endodontics in RVS Dental College and Hospital, Coimbatore, Tamil Nadu, India, from September 2024 to January 2025. This included quality control strains of *C.albicans* and *E.faecalis* obtained from HiMedia laboratories, Bombay, which were cultured in Tryptic soy broth with 5% defibrinated sheep blood for 45 hours under anaerobic conditions at 37°C. The test materials will be divided into the groups as shown in [Table/Fig-1]. Further, the quantity of test drugs poured over filter disc paper via micropipette is 10, 20, 30, and 40 µL.

E.faecalis group

- Group I- Calcium hydroxide (positive control)
- Group II- Saline (negative control)
- Group III- Test drug preparations

The further test group (group III) was divided into three different weighted and mixed ratios at different combinations involving diclofenac, ciprofloxacin, and fluconazole. A sample of three different concentrations was examined across all combinations of drug preparations [Table/Fig-1]. The Institutional Ethics Committee (or Institutional Review Board) was approved before the start of the study (Ref No: 31/ETHICS/2024).



[Table/Fig-1]: Experimental grouping and drug allocation for *E.faecalis* and *C.albicans*.

The API powders of the test drugs were weighed and mixed in a 1:10, 2:10, and 3:10 ratio with EC polymer, and drug preparations were made as shown in [Table/Fig-2].

Drugs	1:10	2:10	3:10
Diclofenac(D)	Diclo – 200 mg + Polymer solution – 20 mL + Excipient	Diclo – 400 mg + Polymer solution – 20 mL + Excipient	Diclo – 600 mg + Polymer solution – 20 mL + Excipient
Ciprofloxacin (C)	Cipro – 200 mg + Polymer solution – 20 mL + Excipient	Cipro – 400 mg + Polymer solution – 20 mL + Excipient	Cipro – 600 mg + Polymer solution – 20 mL + Excipient
Diclofenac + Ciprofloxacin (DC)	Diclo – 200 mg + Cipro – 200 mg + Polymer solution – 20 mL + Excipient	Diclo – 400 mg + Cipro – 400 mg + Polymer solution – 20 mL + Excipient	Diclo – 600 mg + Cipro – 600 mg + Polymer solution – 20 mL + Excipient
Diclofenac + Ciprofloxacin + Fluconazole (DCF)	Diclo – 200 mg + Cipro – 200 mg + Fluc – 200 mg + Polymer – 20 mL + Excipient	Diclo – 400 mg + Cipro – 400 mg + Fluc – 400 mg + Polymer – 20 mL + Excipient	Diclo – 600 mg + Cipro – 600 mg + Fluc – 600 mg + Polymer – 20 mL + Excipient

[Table/Fig-2]: Test drugs- against *E.faecalis*.

C.albicans group

- Group I- Calcium hydroxide (positive control)
- Group II- Saline (negative control)
- Group III- Test drug preparations

API powders of the test drugs were weighed and mixed in 1:10, 2:10, and 3:10 ratios with EC polymer, and drug preparations were made as shown in [Table/Fig-3].

The EC is a hydrophobic polymer used to retard drug release in sustained-release formulations. Lower drug-to-polymer ratios (e.g.,1:10) create denser matrices, slowing diffusion and release. Higher ratios (e.g., 3:10) allow faster release, as the polymer barrier is thinner relative to drug content [11].

Accurate measurement of the growth inhibition zone size in antimicrobial susceptibility tests is crucial for laboratory technicians, yet it can be extremely time consuming and labour-intensive. Overlapping zones frequently occur, increasing the likelihood of

measurement errors. HiMedia's original invention, the zone scale, provides an effective solution to this problem.

1. Placing the plate on some dark surface, slide the scale on the inhibition zone to be measured to match the appropriate circle on the scale and read. Write the measured size on the plate with a marker pen.
2. Measure all the inhibition zones in the above manner and write the sizes measured on the corresponding zones.
3. It is suggested that the zone scale be kept in its resealable PP transparent case to ensure that the scale stays scratch-free. PW096, an antibiotic zone scale of dimensions 370×65 mm, is a convenient means of accurate zone reading.
4. It can measure zones in the range of 10-40 mm. PW297 is a compact (packet size) antibiotic zone reading scale of dimensions 200×95 mm. The zone scale can measure sizes of zones in the range of 10-40 mm [12].

Drugs	1:10	2:10	3:10
Diclofenac (D)	Diclo – 200 mg + Polymer solution – 20 mL + Excipient	Diclo – 400 mg + Polymer solution – 20 mL + Excipient	Diclo – 600 mg + Polymer solution – 20 mL + Excipient
Fluconazole (F)	Fluco– 200 mg + Polymer solution – 20 mL + Excipient	Fluco– 400 mg + Polymer solution – 20 mL + Excipient	Fluco– 600 mg + Polymer solution – 20 mL + Excipient
Diclofenac + Fluconazole (DF)	Diclo – 200 mg + Fluco– 200 mg + Polymer solution – 20 mL + Excipient	Diclo – 400 mg + Fluco– 400 mg + Polymer solution – 20 mL + Excipient	Diclo – 600 mg + Fluco– 600 mg + Polymer solution – 20 mL + Excipient
Diclofenac + Ciprofloxacin + Fluconazole (DCF)	Diclo – 200 mg + Cipro – 200 mg + Fluc – 200 mg + Polymer – 20 mL + Excipient	Diclo – 400 mg + Cipro – 400 mg + Fluc – 400 mg + Polymer – 20 mL + Excipient	Diclo – 600 mg + Cipro – 600 mg + Fluc – 600 mg + Polymer – 20 mL + Excipient

[Table/Fig-3]: Test drugs- against *C.albicans*.

The antimicrobial activity of the mentioned groups was tested against *E.faecalis* and *C.albicans* using the disk diffusion method [Table/Fig-4-8]. A microbial lawn was prepared on Mueller-Hinton by spreading 100 µL of a suspension containing 1.5×10^8 CFU/mL of *E.faecalis* or *C.albicans* in Mueller-Hinton growth medium. Disks with a diameter of 6 mm were aseptically punched into the agar and filled with exactly 10, 20, 30, 40 µL of the test material. The plates were then incubated at 37°C for 24 hours, and the diameter of the zone of inhibition was measured using the Hi Antibiotic Scale.

STATISTICAL ANALYSIS

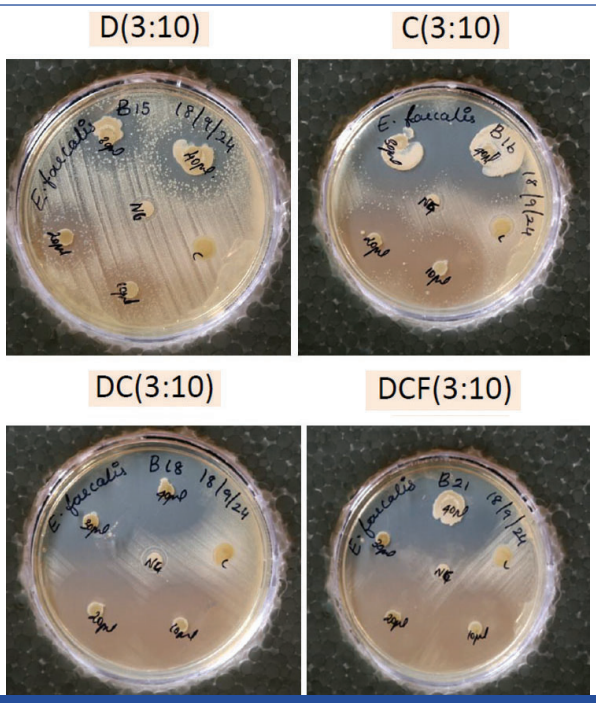
Data was collected using a Microsoft Excel spreadsheet and analysed using SPSS version 24.0. Basic descriptive statistics for the antimicrobial activity of all three groups against *E.faecalis* and *C.albicans* were calculated, including the mean and standard deviation. Inter-group comparisons were performed using One-way ANOVA, with the significance level set at 0.05.

RESULTS

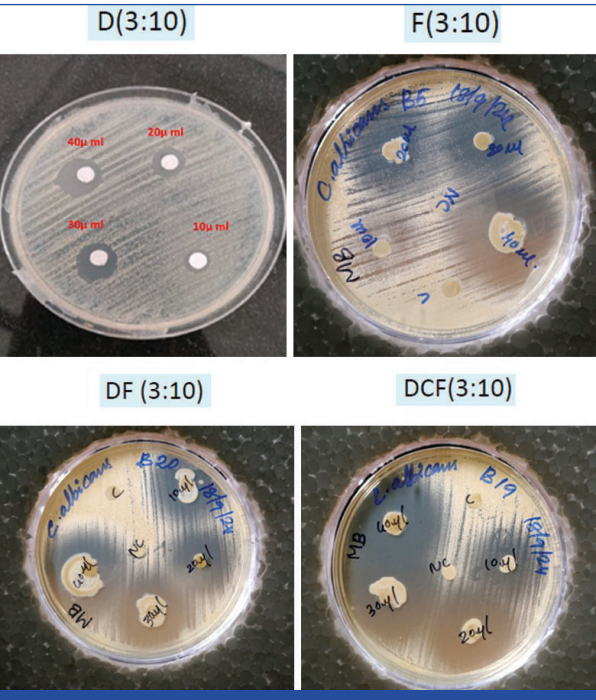
The results indicate that the DCF (3:10) formulation exhibited the highest antimicrobial activity against both *E.faecalis* and *C.albicans*, showing significantly larger inhibition zones [Tables/Fig-9,10]. Intergroup comparisons among all three groups revealed a significant difference in the inhibition of both *E.faecalis* and *C.albicans* (p-value <0.001), as shown in [Tables/Fig-11,12], respectively.

For *E.faecalis*, the combination of Diclofenac, Ciprofloxacin, and Fluconazole (3:10) demonstrated the largest zone of inhibition, with a mean measurement of 35.75 mm. The efficacy was ranked as follows: Diclofenac, Ciprofloxacin, Fluconazole > Diclofenac, Ciprofloxacin > Ciprofloxacin > Diclofenac > $Ca(OH)_2$ > Saline.

[Table/Fig-4]: Stock culture of *E.faecalis* and *C.albicans*.



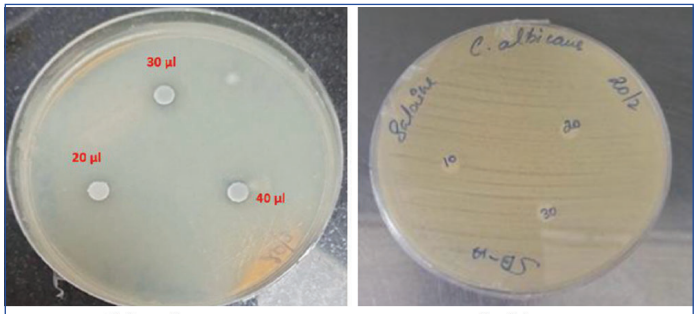
[Table/Fig-5]: Zone of inhibition for *E.faecalis*.



[Table/Fig-6]: Inhibition zone for *C.albicans*.

E.faecalis *C.albicans*

[Table/Fig-7]: $Ca(OH)_2$ - Inhibition zone.



E.faecalis *C.albicans*

[Table/Fig-8]: Saline – inhibition zone.

Groups		10 µL	20 µL	30 µL	40 µL
Group III	D (1:10)	0	0	0	0
	C (1:10)	17	18	20	20
	DC (1:10)	22	22	24	25
	DCF (1:10)	30	31	31	33
	D (2:10)	5	6	6	7
	C (2:10]	18	19	20	21
	DC (2:10)	24	24	25	26
	DCF (2:10)	32	32	34	35
	D (3:10)	11	14	16	17
	C (3:10)	19	19	21	22
	DC (3:10)	25	26	26	28
	DCF (3:10)	34	35	36	38*
Group I $CA(OH)_2$		9	12	15	16
Group II Saline		0	0	0	0

[Table/Fig-9]: Inhibition zone (mm). - *E.faecalis*.
µL - microliter of test solution; *- highest zone of inhibition; D: Diclofenac; C: Ciprofloxacin; DC: Diclofenac + Ciprofloxacin; DCF: Diclofenac + Ciprofloxacin + Fluconazole

Groups		10 µL	20 µL	30 µL	40 µL
Group III	D(1:10)	0	0	0	0
	F(1:10)	15	17	17	19
	DF(1:10)	19	20	21	22
	DCF(1:10)	24	28	28	29
	D(2:10)	4	5	5	7
	F(2:10)	16	17	18	20
	DF(2:10)	19	21	23	24
	DCF(2:10)	25	28	29	31
	D(3:10)	9	12	13	15
	F(3:10)	19	21	22	23
	DF(3:10)	21`	23	24	25
	DCF(3:10)	28	29	32	35*
Group I $Ca(OH)_2$		8	12	14	16
Group II Saline		0	0	0	0

[Table/Fig-10]: Inhibition zone (mm).- *C.albicans*.
µL: microliter of test solution; *- highest zone of inhibition; D: Diclofenac; F: Fluconazole; DF: Diclofenac + Fluconazole; DCF: Diclofenac + Ciprofloxacin + Fluconazole

Groups		N	Mean	Std. Deviation	F value	p-value
Group III	D(1:10]	4	.0000	.00000	205.609	<0.001**
	C(1:10]	4	18.7500	1.50000		
	DC(1:10]	4	23.2500	1.50000		
	DCF(1:10]	4	31.2500	1.25831		
	D(2:10)	4	6.0000	.81650		
	C(2:10)	4	19.5000	1.29099		
	DC(2:10)	4	24.7500	.95743		
	DCF(2:10)	4	33.2500	1.50000		
	D(3:10)	4	14.5000	2.64575		
	C(3:10)	4	20.2500	1.50000		
	DC(3:10)	4	26.2500	1.25831		
	DCF(3:10)	4	35.7500a	1.70783		
Group I-$Ca(OH)_2$		4	13.0000	3.16228		
Group II-Saline		4	0	0		

[Table/Fig-11]: Intergroup comparison for *E.faecalis*.
**highly Significant <0.001; a: the highest mean of inhibition; D: Diclofenac; C: Ciprofloxacin; DC: Diclofenac + Ciprofloxacin; DCF: Diclofenac + Ciprofloxacin + Fluconazole

Groups		N	Mean	Std. Deviation	F value	p-value
Group-III	D(1:10)	4	.0000	.00000	93.913	0.001**
	F(1:10)	4	17.0000	1.63299		
	DF(1:10)	4	20.5000	1.29099		
	DCF(1:10)	4	27.2500	2.21736		
	D(2:10)	4	5.2500	1.25831		
	F(2:10)	4	17.7500	1.70783		
	DF(2:10)	4	21.7500	2.21736		
	DCF(2:10)	4	28.2500	2.50000		
	D(3:10)	4	12.2500	2.50000		
	F(3:10)	4	21.2500	1.70783		
	DF(3:10)	4	23.2500	1.70783		
	DCF(3:10)	4	31.0000a	3.16228		
Group I-$Ca(OH)_2$		4	12.5000	2.41565		
Group III-Saline		4	.0000	.00000		

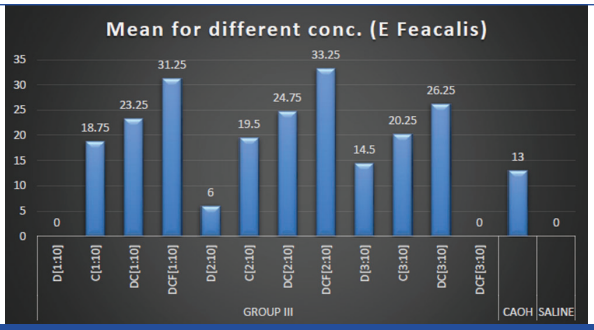
[Table/Fig-12]: Intergroup comparison for *C.albicans*.
**highly Significant <0.001; a: the highest mean of inhibition; D: Diclofenac; F: Fluconazole; DF: Diclofenac + Fluconazole; DCF: Diclofenac + Ciprofloxacin + Fluconazole

Similarly, against *C.albicans*, the combination of Diclofenac, Ciprofloxacin, and Fluconazole (3:10) again showed superior activity, with a mean zone of inhibition of 31.00 mm, outperforming other combinations, including Diclofenac fluoride, Fluconazole, and individual drug preparations. [Table/Fig-13,14] illustrates the mean inhibition zones for *E.faecalis* and *C.albicans*, respectively.

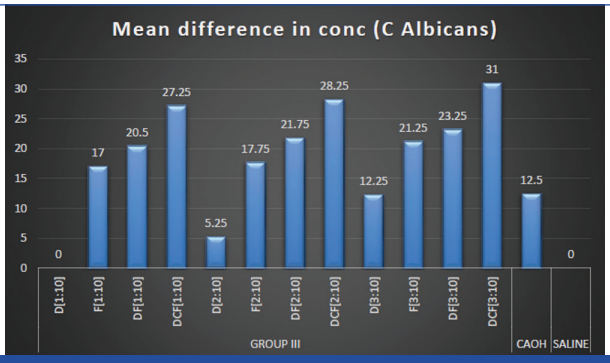
DISCUSSION

The present study discussed the efficacy of modified novel double antibiotic preparations against *E.faecalis* and *C.albicans*. The test drugs included ciprofloxacin, fluconazole, and diclofenac in varying ratios. Ciprofloxacin, a broad-spectrum fluoroquinolone antibiotic, inhibits bacterial DNA gyrase, leading to bacterial cell death. Fluconazole, an antifungal agent, disrupts ergosterol synthesis, a crucial component of fungal cell membranes. Diclofenac, an NSAID, has demonstrated antimicrobial and antibiofilm properties, particularly in combination therapies. This study proved that the 3:10 ciprofloxacin, fluconazole, and diclofenac preparation was effective against *E.faecalis* and *C.albicans*.

Enterococcus faecalis is a prominent multi-resistant pathogen associated with nosocomial infections and is the most commonly isolated species from persistently infected dental root canals. This indicates that the oral cavity may serve as a reservoir for



[Table/Fig-13]: Mean distribution of *E.faecalis* intergroup comparison.



[Table/Fig-14]: Mean distribution of *C albicans* intergroup comparison.

resistant strains. The existence of virulence factors can lead to the ineffectiveness of standard endodontic treatments [13]. Mutations in gene regulation and the activation of particular regulatory genes play a significant role in this process. *C.albicans* can adhere to dentin and establish biofilms, which are naturally resistant to antifungal medications, the host's immune response, and various environmental stressors, presenting a significant clinical challenge [14].

This study supports the alternative hypothesis, indicating that the antimicrobial efficacy of the combinations of Diclofenac, Ciprofloxacin, and Fluconazole with EC is superior against *Enterococcus faecalis* and *Candida albicans* when compared to calcium hydroxide.

In the case of *E.faecalis*, the DCF (3:10) preparation exhibited the highest antimicrobial activity, as evidenced by larger inhibition zones compared to other groups. The positive control, calcium hydroxide, exhibited moderate activity, while the negative control, saline, showed no inhibition. The statistical analysis revealed a significant reduction in bacterial growth (p-value <0.001) with DCF (3:10), confirming its superior efficacy in targeting resistant bacterial strains. Similarly, the effectiveness of DCF (3:10) against *C.albicans* was noteworthy. DCF (3:10) produced significantly larger inhibition zones compared to other drug preparations, as disk as the positive and negative controls. Statistical analysis further confirmed that the interventional groups, particularly DCF (3:10), significantly reduced fungal growth (p-value <0.001).

The use of an EC polymer as a delivery vehicle likely enhanced the drug's release profile and bioavailability, contributing to the observed antimicrobial activity. The synergistic effects of diclofenac and ciprofloxacin in DCF formulations provided enhanced efficacy by disrupting biofilms and targeting microbial cell wall integrity. This finding underscores the importance of optimising drug concentrations and combinations to maximise therapeutic outcomes [11,15].

Ferrer-Luque CM et al., (2023) demonstrated that diclofenac, in combination with antibiotics, exhibits significant antibiofilm activity, enhancing its potential as an endodontic intracanal medicament [16]. Their study highlighted that diclofenac disrupts biofilms by inhibiting microbial adherence, making it a promising adjunctive agent in endodontic therapy Hence, it support the present study's findings, further validating the antimicrobial potential of diclofenac-based formulations in endodontic disinfection.

In endodontics, effective pain management is sometimes challenging. The efficacy of Dexamethasone Sodium Phosphate (DCS) using different delivery routes for preventing post-endodontic pain has been studied with favourable results [16,17]. Likewise, studies have demonstrated the antimicrobial efficacy of DCS, considering it a non antibiotic compound useful in resistant infections of various kinds. Diclofenac sodium (D) was found to possess antibacterial activity against both drug-sensitive and drug-resistant clinical isolates of *Staphylococcus aureus, Listeria monocytogenes, Escherichia coli*, and *Mycobacterium spp*., in addition to its potent anti-inflammatory activity [18].

Lo WH et al., reported that fluconazole combined with ciprofloxacin can inhibit the growth of *C.albicans* by disrupting ergosterol synthesis, which plays a critical role in the tolerance of *C.albicans* to antifungal agents [19]. The coordination and irreversibility of cell cycle phases are crucial for cellular morphogenesis in *C.albicans*. The combination of ciprofloxacin and fluconazole demonstrated the most effective activity against *C.albicans*. Additionally, local drug delivery targeting *C.albicans* and *E.faecalis* suggests that this combination is an optimal therapeutic recommendation.

Fluconazole demonstrates a synergistic effect with Diclofenac, hence diclofenac (D) enhances the efficacy of these azole medications in combating biofilm formation [19]. Additionally, fluconazole may exhibit increased effectiveness when paired with a drug that mitigates resistance in *C.albicans*. Helper compounds and macrophage modulators enhance the cytotoxic activity of macrophages that have engulfed microorganisms. Locally administered diclofenac proves to be more effective than systemic administration, exhibiting reduced tissue toxicity [16].

The potential implementation of endodontic therapy alongside an innovative antibiotic combination with other medications aims to improve disinfection during root canal procedures and alleviate postoperative discomfort. Literature has identified several benefits of using antibiotics as the preferred intracanal medicament, particularly highlighting their significant alkalinity, capacity to dissolve tissue, effectiveness in neutralising endotoxins, and antibacterial characteristics [20,21].

For instance, after seven days, diclofenac lowered the pH of the paste while sustaining a more potent antimicrobial effect. It also indicated that the antimicrobial properties are not solely dependent on the paste's alkalinity. Diclofenac sodium demonstrates strong bactericidal activity against both gram-positive and gram negative bacteria by interfering with bacterial DNA synthesis [22].

The presence of DS significantly improved the initial dissolution rate of ciprofloxacin in a phosphate buffer, achieving a maximum of 80%. However, the percentage dissolved decreased to around 20% by the end of the testing period. The increased bioavailability of ciprofloxacin when co-administered with DS is attributed to the formation of an ion pair complex [23]. These findings highlight the importance of optimising drug concentrations and combinations to maximise therapeutic outcomes [23].

The present study revealed that antibiotics combined with anti-inflammatory drugs were more effective against and *C.albicans*. The data indicate that certain NSAIDs show promise as drug candidates for developing dual-action medications aimed at treating both infectious and inflammatory diseases.

By optimising drug formulations to enhance their half-life, we can significantly improve the therapeutic efficacy of these agents. Such advancements could lead to a more localised treatment approach, thereby reducing systemic side effects and minimising the risk of off-target exposure. This is especially crucial in managing complex infectious and inflammatory conditions, where current treatments often fail to provide optimal results. Enhanced targeted therapies not only promise improved patient outcomes but also pave the way for more personalised medicine strategies.

Limitation(s)

Further investigation is essential to thoroughly understand the dual mechanisms of action exhibited by these agents, which integrate both anti-inflammatory and antimicrobial properties. This deeper exploration could elucidate how these mechanisms interact at the cellular and molecular levels, potentially revealing new therapeutic pathways. Moreover, a comprehensive study of targeted drug delivery systems is warranted.

CONCLUSION(S)

The association between infectious agents and chronic inflammatory diseases has significant implications for public health, treatment, and prevention efforts. Treating conditions that involve both infections and inflammation often requires the use of multiple medications, including antibiotics and anti-inflammatory drugs. In the current study, the DCF (3:10) anti-inflammatory combination demonstrated the highest antimicrobial efficacy against both *E.faecalis* and *C.albicans*. Therefore, it can be considered a potential intracanal medicament for these pathogens. However, future clinical trials are necessary to confirm the antibacterial effects of these NSAIDs before they can be practically applied in treatments.

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