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ASSESSING THE ANTIMICROBIAL POTENTIAL OF NON-ANTIBIOTIC DRUGS USED AS AN INTRACANAL MEDICAMENT AGAINST E.FAECALIS : AN INVITRO STUDY

Endodontics

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ABSTRACT

Conventional intracanal medicaments such as calcium hydroxide and triple antibiotic paste have limitations including reduced efficacy against E. faecalis and antibiotic resistance respectively. These concerns have led to evaluation of non-antibiotic drugs with antimicrobial properties like Metformin as intracanal medicament. **Aim:** To compare the antimicrobial effect of triple antibiotic paste (TAP), metformin, calcium hydroxide, and metformin–calcium hydroxide combination against Enterococcus faecalis.**Methods and Material:** An in-vitro study was conducted using the agar well diffusion method. Four groups were prepared: Group A – TAP, Group B – Metformin, Group C – Calcium hydroxide, and Group D – Metformin with calcium hydroxide. Enterococcus faecalis ATCC 51299 was cultured on Mueller–Hinton agar plates. The finely powdered medicaments were introduced into the wells prepared in the agar medium. After 24-hour incubation at 37°C, zones of inhibition were measured in millimeters. **Statistical analysis:** The data obtained were analysed using one-way ANOVA followed by post hoc Tukey's multiple comparison test. A p-value < 0.05 was considered statistically significant.**Results:** TAP demonstrated the greatest zone of inhibition against E. faecalis, indicating the highest antimicrobial efficacy. Metformin showed better antibacterial activity than calcium hydroxide alone and the combination of Metformin–Ca $(OH)_2$. There was no significant change in the pH values of Ca $(OH)_2$ paste in combination with Metformin and calcium hydroxide alone**Conclusions:** TAP showed the highest antibacterial activity against E. faecalis. The non-antibiotic drug Metformin provided efficient antimicrobial effect than calcium hydroxide. Addition of calcium hydroxide with metformin provided no significant antimicrobial effect on E.faecalis.

KEYWORDS

Enterococcus Faecalis, Triple Antibiotic Paste, Metformin, Calcium Hydroxide, Metformin-calcium Hydroxide .

INTRODUCTION:

Modern endodontics emphasizes single-visit endodontic treatments comprising the entire cleaning, shaping, and obturation of canals performed in a single appointment [1]. However, the management of necrotic teeth, persistent apical periodontitis, and apical abscess with continuous exudation and internal resorption requires multiple-visit root canal inter-appointments with intracanal disinfection [2]. The complex root canal anatomy, polymicrobial nature of root canal infection, and penetration of biofilms into deep radicular dentinal tubules make single-visit endodontics insufficient for managing the abovementioned conditions. Intracanal disinfection with medicaments, mainly calcium hydroxide and triple antibiotic paste (TAP), is extensively needed as adjuvant agents between appointments to reduce microbial load, inflammation, and promote healing of periapical pathological conditions.

Calcium hydroxide has been the material of choice among intracanal medicaments because of its high alkaline pH of 12.5, which renders superior antimicrobial activity and reduced cytotoxicity to the peri radicular tissues [3]. With the advent of regenerative endodontics, the use of triple antibiotic paste, which is a combination of ciprofloxacin, metronidazole, and minocycline, as an intracanal medicament was recommended for disinfection [4]. However, calcium hydroxide is less effective against Enterococcus faecalis as it can survive the alkalinity of calcium hydroxide.[5] Calcium hydroxide also reduces the fracture resistance of radicular dentine. High concentrations of TAP provide efficient antimicrobial activity but are cytotoxic to the apical papilla. They also reduce dentine microhardness, flexural strength, and increase antimicrobial resistance [6,7]. Considering these disadvantages of existing intracanal medicaments, increased attention has been focused on biocompatible bioactive formulations and effective alternatives.

Advanced bioinformatic studies have exploited the repurposing of non-antibiotic drugs, which provide an alternative strategy to break antimicrobial resistance. Non-antibiotic drugs such as NSAIDs, antidepressants, antipsychotics used to treat non-infectious diseases, have been shown to exhibit antimicrobial properties and explored for use as intracanal medicament [8,9]. Recently, Metformin, a commonly used drug for the treatment of type 2 diabetes mellitus, has been repurposed as adjuvant antibiotics [10]. Wang et al. studied the use of Metformin in root canals associated with apical periodontitis and observed healing of bone resorption [11].In order to improve the action of calcium hydroxide against E. faecalis, it is used in conjunction with other therapeutic agents. Liu et al. studied that metformin combined with Calcium hydroxide reduces the alkaline resistance of E. faecalis against calcium hydroxide [12]. Comparative evaluation of the antimicrobial effect of Metformin, the metformin and calcium hydroxide combination, triple antibiotic paste, and calcium hydroxide remains unexplored. The aim of this study is to compare the antimicrobial activity of triple antibiotic paste, Metformin, calcium hydroxide, and the combination of Metformin and calcium hydroxide against E. faecalis. As alkalinity is considered an important parameter for the antimicrobial property of calcium hydroxide, the influence of metformin on the pH in combination was also studied.

Materials and Methods:

Preparation of medicaments:

The study was performed for a total of 24 samples. Four test groups were allocated with 6 samples in each group.

Group A: TAP (1:1:1 w/v) – prepared by mixing metronidazole (Vikrams metronidazole 400), ciprofloxacin (CIPRODAC 500), and minocycline (MINOZ 50). The capsules or coating materials of the drug were removed , pulverized into fine powders using autoclavable mortars and pestles and mixed with distilled water (1 mg/ml).

Group B: Metformin (Okamet 500) (1:1 w/v) dissolved in Dimethyl Sulfoxide (DMS) and mixed with distilled water.

Group C: Calcium hydroxide (PRIME 10g) (1:1 w/v) mixed with distilled water.

Group D: Metformin and Calcium hydroxide (1:1 w/v). Powdered form of metformin dissolved in Dimethyl Sulfoxide (DMS) was mixed with calcium hydroxide powder and distilled water.

The pH of each group was evaluated using pH meter before the introduction of medicament in the agar plates.

Bacterial strains and media: The E. faecalis strain ATCC 51299 was acquired. The strains were isolated from a blood agar plate inoculated for 24 hours at 37 degrees centigrade in the incubator.

Agar well diffusion assay: A total of 8 MH plates were utilized, with two MH agar plates assigned for each group. Each Mueller–Hinton agar (MH) plates were containing 3 wells, 5 mm in diameter and 2 mm in depth. 30 µl of the drug to be tested was dispensed into each well. Uniform distribution of the cultured bacteria on the agar plates was achieved using cotton swabs. The plates were aerobically incubated at 37°C for 24 hours. Following incubation, the inhibition zone was measured by a blinded examiner (Figure 1) and tabulated. The mean zone of inhibition of each group was calculated.

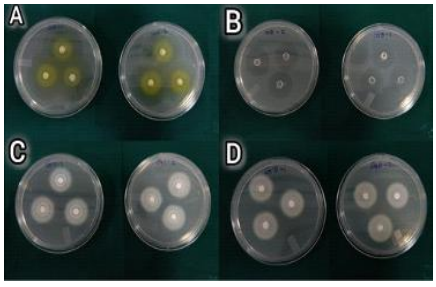


Figure 1 Antimicrobial activity assessment using Mueller Hinton agar against E.faecalis

Zone of inhibition exhibited by A)TAP (Group A). B) Metformin(Group B).C) Calcium hydroxide (Group C) D) Metformin +Calcium hydroxide (Group D).

RESULTS:

The obtained data were analysed using one-way ANOVA and post-hoc Tukey test to compare the diameter of the growth inhibition zone across different groups. A significance level of p-value less than 0.05 was considered statistically significant.

Table 1 shows the mean zone of inhibition and standard deviation of the tested groups against E. faecalis. Figure 1 shows the zone of inhibition of the tested groups. Group A (TAP) exhibited the highest zone of inhibition [3.4667], suggesting a highly efficient antimicrobial effect against E. faecalis. Group B (Metformin) showed increased efficacy, followed by TAP with a mean zone of inhibition of 2.4500. The least antimicrobial efficacy against E. faecalis was exhibited by Group C and Group D.

Table 1 shows the pH of the tested intracanal medicament. Tap and metformin exhibited a pH of 5.08 and 6.73 respectively. Calcium hydroxide and the combination of calcium hydroxide + Metformin showed almost similar alkaline pH 12.43 and 12.34 respectively. As shown in Table 2, TAP exhibited a statistically significant highest mean zone of inhibition among all the tested groups. Metformin exhibited significant increased antimicrobial efficacy than Calcium hydroxide and Group D (Calcium hydroxide + Metformin). Calcium hydroxide exhibited a statistically significant lower mean zone of inhibition than TAP and Metformin. There was no significant difference among the zone of inhibition values of Group C and Group D. Figure 2 presents the graphical representation of the antimicrobial efficacy of different groups against E. faecalis.

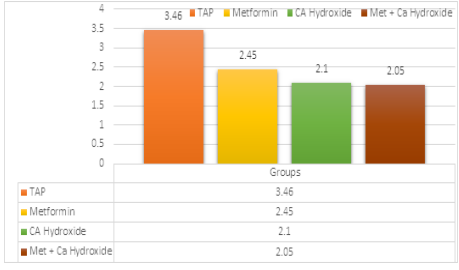
Table1:Mean zone of inhibitions (mm) and pH value of the tested groups against E.faecalis

Groups	N	pH	Mean	Std.Deviation	P Value
Control	3	5.08	0	0	0.05*
Group A:TAP	6	6.73	3.4667	.25820	
Group B:Metformin	6	12.43	2.4500	.12247	
Group C:Calcium Hydroxide	6	12.34	2.1000	.10954	
Group D:Metformin + Calcium Hydroxide	6	5.08	2.0500	.12247	

Table 2: Intergroup comparison of the antimicrobial efficacy of the tested groups on E.faecalis

(I) Group A	(J) Group B	Mean Difference (I-J)	Sig.
Control	TAP	-3.46667*	.003
	Metformin	-2.45000*	.004
	Ca Hydroxide	-2.10000*	.009
	Met + Ca Hydroxide	-2.05000*	.008
TAP	Control	3.46667*	.005
	Metformin	1.01667*	.007
	Ca Hydroxide	1.36667*	.001
	Met + Ca	1.41667*	.003
Metformin	Control	2.45000*	.004
	TAP	-1.01667*	.002
	Ca Hydroxide	.35000*	.007
	Met + Ca Hydroxide	.40000*	.002
Calcium Hydroxide	Control	2.10000*	.006
	TAP	-1.36667*	.005
	Metformin	-.35000*	.007
	Met + Ca Hydroxide	.05000	.981
Metformin + Calcium Hydroxide	Control	2.05000*	.004
	TAP	-1.41667*	.008
	Metformin	-.40000*	.002
	Ca Hydroxide	-.05000	.981

Figure 2 :Graphical representation of antimicrobial efficiency of different groups against E.faecalis



DISCUSSION:

Metformin is a commonly known drug for the treatment of type 2 diabetes mellitus. The antihyperglycemic action is due to the inhibition of hepatic gluconeogenesis, while a secondary mechanism involves the enhancement of insulin sensitivity in peripheral tissues and glucose absorption in the gut [13]. Drug repurposing or repositioning has established new indications for the already known antihyperglycemic drug Metformin. Recent studies indicated that topical application of metformin effectively improves the periodontal parameters of patients with diabetes and periodontitis, enhances periodontal regeneration, bone integration of endoosseous implants, and improves tooth movement in orthodontics [14]. Moreover, studies suggested that metformin stimulates odontogenic differentiation, mineral synthesis of stem cells in the tooth pulp and pre-conditioning with metformin enhances the angiogenic potential of dental pulp stem cells [15,16]. Wang et al. suggested that metformin used as intracanal medicament promoted the healing of apical periodontitis [17]. Metformin is evaluated in the current study based on this drug repurposing concept, which may help reduce antibiotic resistance and expand the range of effective medicaments for disinfecting canals in regenerative endodontic procedures and persistent endodontic treatments.

Enterococcus faecalis (E. faecalis), a Gram-positive bacterium, is frequently responsible for endodontic treatment failures leading to persistent apical periodontitis [18]. Calcium hydroxide, the most commonly used intracanal medicament, is ineffective against E. faecalis. The use of TAP against E.faecalis may result in antibiotic resistance. Based on studies on eradicating E.faecalis, an approach that combines anti-biofilm agents and antimicrobial drugs stands effective [19]. Hence, a combination of calcium hydroxide and metformin was evaluated in managing E.faecalis. The agar well diffusion assay (Kirby–Bauer method) is a simple in-vitro method that is reliable for the assessment of antimicrobial activity of intracanal medicaments.

The antimicrobial efficacy was evaluated by measuring the zone of inhibition around the wells or disc as shown in Figure 1 [20].

In the present study, the inter group comparison showed that TAP exhibited a statistically significant highest mean zone of inhibition when compared with all other groups, with a mean inhibition zone value of 3.4667. This suggests that TAP exhibited the highest antimicrobial effect on E. faecalis compared to the other tested groups. This is in accordance with previous studies that suggest that TAP can be effectively used for root canal disinfection and healing of periapical diseases [21]. This is because of the broad-spectrum antimicrobial action of the nitroimidazole compound metronidazole, which is effective against protozoa and anaerobic bacteria, semisynthetic tetracycline minocycline, and a synthetic bactericidal fluoroquinolone ciprofloxacin.

Metformin (Group B) exhibited significantly increased antimicrobial efficacy compared to calcium hydroxide (Group C) and (Group D) calcium hydroxide + metformin combination. This is in accordance with studies that suggest that the intracanal application of metformin improves the healing of periapical pathosis by suppressing inducible nitric oxide synthase and monocyte recruitment [17]. Liu et al. studied that metformin attenuates alveolar bone destruction with apical periodontitis and inhibits pro-inflammatory cytokine synthesis [22].
Calcium hydroxide exhibited a statistically significant lower mean zone of inhibition than TAP and Metformin. This is consistent with previous studies that state that calcium hydroxide was ineffective in the destruction of E. faecalis biofilms but contributed to the biofilm structure.[23] According to Chávez de Paz et al and Weckwerth et al., E. faecalis can tolerate the alkaline pH range of 9-11 of calcium hydroxide [23,24].

The combination of Metformin + Calcium Hydroxide [2.0500] showed decreased antimicrobial efficacy than calcium hydroxide alone. This suggests that combining Metformin with $Ca(OH)_2$ does not exert a synergistic effect. This contradicts the result obtained by Liu et al, which showed that metformin boosts the antimicrobial action of calcium hydroxide against E. faecalis [12]. This can be explained from the pH evaluation results obtained. Calcium hydroxide exhibited pH 12.43. Calcium hydroxide combined with Metformin achieved a pH of 12.34. There was no significant change in the pH values of $Ca(OH)_2$ paste in combination with Metformin and calcium hydroxide alone. The addition of metformin with calcium hydroxide did not alter the pH, which explains its least antibacterial action.

This in-vitro study possesses the limitation of not completely simulating the clinical polymicrobial infection of the root canal, as antimicrobial efficacy was evaluated only against Enterococcus faecalis. The penetration into dentinal tubules or activity against mature biofilms are not evaluated. Only selected concentrations of the non-antibiotic drugs were tested. Considering the clinical use of metformin, future studies on effective concentrations, effect on radicular dentin, and their biocompatibility with periapical tissues should be assessed to optimize treatment protocols.

CONCLUSION:

Triple Antibiotic Paste (TAP) exhibited the highest antimicrobial action against E. faecalis. Metformin showed increased antimicrobial efficacy as non -antibiotic intracanal medicament. The combination of calcium hydroxide with Metformin did not enhance the antimicrobial property of calcium hydroxide. Calcium hydroxide and the Metformin–$Ca(OH)_2$ combination provided the least antimicrobial efficacy against E. faecalis when compared with Metformin and Triple antibiotic paste.

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