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RESEARCH ARTICLE

ANTIBACTERIAL EFFICACY OF CLINDAMYCIN GEL - INCORPORATED OBTURATING MATERIALS FOR PRIMARY TEETH AGAINST ENTEROCOCCUS FAECALIS – AN IN - VITRO STUDY

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ABSTRACT

Aim and objective: To assess the Antibacterial efficacy of clindamycin gel incorporated Zinc oxide eugenol and Endoflas FS, two obturating materials used in pulpectomy, against enterococcus faecalis in primary teeth. ***Materials and methods:*** The study consisted of four groups, with 11 samples in each group: group I (zinc oxide eugenol paste), group Ia (Zinc oxide eugenol paste + 2% Clindamycin gel), group II (Endoflas FS), and group IIa (Endoflas FS+ 2% clindamycin gel). A double layer agar well diffusion test was used to evaluate the antibacterial activity against *enterococcus faecalis*. The zones of microbial inhibition were measured at the end of 24 hours, 6th day, and 29th day. ***Results:*** On intergroup comparison, the difference in the antibacterial activity was found to be highly significant (p <0.001). Among the various groups evaluated, group IIa showed the highest antibacterial activity against *E. faecalis* followed by group II, group Ia, and the least activity being shown by group I throughout the experimental periods. On intragroup comparison at different time intervals, a maximum zone of inhibition was seen at 24 hours with a p value < 0.05 in all the tested groups. ***Conclusion:*** Incorporation of 2 % Clindamycin gel into Zinc oxide eugenol and Endoflas FS enhanced the antimicrobial activity of both the root canal filling materials with lasting antimicrobial activity even at the end of the 29th day. ***Clinical significance:*** An essential prerequisite for a root canal filling material is its antibacterial efficiency, which will aid in eliminating residual microorganisms in the root canal system following chemo mechanical preparation. A pulpectomy-treated tooth has a better prognosis when the root canal filling materials' antibacterial efficiency is greatly increased when an antimicrobial agent, like clindamycin gel, is added to them.

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INTRODUCTION

Paediatric endodontics is an integral part of paediatric dentistry which aims to preserve fully functional primary teeth in the dental arch. Despite repeated emphasis on prevention of caries in primary teeth, the occurrence of pulpally involved primary teeth continues to be common problem. [1]Therapeutic approach mainly remains curative in nature. Pulp therapy of primary teeth constitutes a great challenge to paediatric dentists due to complex anatomy of the canal system and change their morphology with root resorption which make microorganisms proliferate and invade lateral canals, apical deltas and dentinal tubules.[2] The effectiveness of instrumentation on bacteria in dentinal tubules and lateral canal ramifications in primary teeth is limited. The key to long-term success of paediatric endodontic therapy is directly related to the reduction and elimination of the infecting bacteria inside root canal system.[3] Enterococcus faecalis are the most common bacterial species that have been detected in the failure of root canal treatment in primary teeth.

E. *faecalis* are considered the most resistant strain in dental infection. [4] In spite of chemo mechanical preparation and copious irrigation of canal, there are chances of failure of pulp therapy in primary teeth due to the entrapped microorganisms in the canal space due to the tortuous and complex nature of the root canal system.[5] Thus, for optimal success of endodontic treatment, substances with antimicrobial properties are advocated as obturating materials in deciduous teeth. The obturating material must fulfil the accepted triad of treatment goals, namely removal of diseased tissue, elimination or reduction of bacteria and prevention of canal reinfection. Various materials have been used for the obturation of primary teeth. ZOE was the traditional obturation material in primary dentition. The shortcoming that has restricted it's use of this compound is its low resorption capacity, due to which zinc oxide and eugenol particles are left within the periapical tissues as the physiological root resorption occurs. [6,7] It can irritate the periapical tissues, can cause necrosis of bone and cementum and may alter the path of eruption of succedaneous

tooth.[8] Another commercially available product gained wide popularity is Endoflas, is a mixture of ZOE, Calcium hydroxide and Iodoform. Endoflas is a resorbable paste obtained by mixing a powder containing iodoform (40.6%), zinc oxide (56.5%), calcium hydroxide (1.07%), barium sulphate (1.63%) and liquid consisting of eugenol and paramonochlorphenol. Endoflas FS can disinfect dentinal tubules and difficult-to-reach accessory canals that cannot be disinfected or cleaned mechanically. However, the long-lasting antimicrobial activity of this root canal filling material over a period of time has not been proven.

On the other hand, clindamycin, is a drug of choice in odontogenic infections. This antibiotic can fight various anaerobic, facultative, and anaerobic bacteria. The viscous mixing agent can penetrate well into the niches and crocks of primary root canal, and the antibiotic can fight various root canal endodontic pathogens.[9] To overcome the limitations of eugenol in ZOE and Endoflas, Clindamycin gel mixed with Zinc oxide and Endoflas powder. Aim of the present study was to comparatively evaluate the antibacterial efficacy of the root canal filling materials for deciduous teeth such as Zinc oxide eugenol and EndoflasFS when incorporated with 2% Clindamycin gel. The null hypothesis for the study was set as there will not be any difference in the antibacterial activity of the root canal filling materials for deciduous teeth such as Zinc oxide eugenol and Endoflas FS with or without 2% Clindamycin gel.

MATERIALS AND METHODS

This study was an *in vitro* intergroup comparative study, which was initiated after obtaining ethical clearance from the institutional ethics committee. It consisted of four groups: group I: zinc oxide and eugenol paste group Ia: Zinc oxide + 2% Clindamycin gel, group II: Endoflas-FS, and group IIa: Endoflas FS + 2% Clindamycin gel. The obtained sample size per group was 11 and to detect differences among the means at 0.05 significance level.

Preparation of the experimental root canal filling materials
2% of Clindamycin gel was mixed with of Zinc oxide and of Endoflas FS separately in 1:1 ratio. These materials were mixed using a metal spatula and glass slab in increments to get a uniform smooth mixture that represented group Ia and group IIa.

Evaluation of the antibacterial efficacy: The antibacterial activity of the tested root canal filling materials was tested against *enterococcus faecalis.* After administration of local anaesthesia, access opening was done under rubber dam isolation (Figure 1), microbial samples were collected using a sterile paper point by passing it through the root canal of the primary teeth. (Figure 2). Following removal from the canal, the paper point was immediately placed in cryovials with peptone water (Figure 3) and were transferred to microbiology lab. The sample were inoculated on blood agar plates and incubated at 37 °c for 24 hours (Figure 4). Enterococcus group was confirmed through bile esculin test. By using lawn technique, microbial colonies were spread uniformly on Muller Hinton agar media. Then 4 wells of 4 mm in depth and 6mm in diameter are made in each of the agar plates with equal distance from each other. 2 % Clindamycin gel was freshly mixed with experimental material in 1: 1 ratio and was placed into wells of petri dishes. Bacterial colonisation was observed for each material by growth inhibitory zones (clearing of agar) around each obturating material. The most uniform diameter segment of the zone of inhibition will be determined in millimetres by measuring the shortest distance between the outer margin of the well and initial microbial growth after 24 hours. After measurement of the inhibition zone, all samples were removed aseptically and rinsed with sterile deionized water to remove any attached bacteria. Each sample was then stored in sterilized deionized water until day 6. On the 6th day, new agar plates were prepared. Four standardized wells were punched into this new agar plate along with bacterial inoculation with 0.5 ml of the bacterial suspension. The specimens were taken out from the deionized water, placed into the new wells, and then incubated at 37^{o}c for 24 hours. The inhibition zones around the specimens were measured and recorded in millimetres as day 7 value. After performing the measurements, each sample was removed and stored in the sterilized deionized water until day 29. The procedure was repeated with the fresh agar plates inoculated with microorganisms on the 29th day for obtaining inhibition zone dimension of day 30 (Figure 5)

Statistical analysis: Repeated Anova was used for simultaneous multiple group comparison followed by *post hoc* Tukey's test for group-wise comparison.

RESULTS

Table no. 1 – Intra group comparison of all the 4 groups at 3 different time intervals using Anova. The effectiveness of different treatment groups was evaluated by measuring the mean zone of inhibition at 0 hours, 7th day, and 30th day. Compared to the zinc oxide group, the zinc oxide + clindamycin gel group showed a significantly higher mean zone of inhibition at 0 hours (13.50 mm vs 11.75 mm, p=0.00182). However, by the 7th day and 30th day, both groups exhibited a decrease in mean zone of inhibition, with no significant difference observed between the two.Similarly, the endoflas group had a mean zone of inhibition comparable to the zinc oxide group at 0 hours (11.75 mm), but by the 7th day, it showed a higher mean zone of inhibition (9.25 mm, p=0.0126). At the 30th day, the endoflas group maintained a higher mean zone of inhibition compared to the zinc oxide group (7.00 mm vs 6.25 mm), although the difference was not significant. The combination of endoflas and clindamycin gel (endoflas+ clindamycin gel) resulted in a significantly higher mean zone of inhibition at all time intervals compared to both the zinc oxide group and the endoflas group ($p<0.05$). At the 7th day, the mean zone of inhibition was the highest amongst the groups (12.00 mm). Overall, the results indicate that the combination of endoflas and clindamycin gel showed the greatest effectiveness in inhibiting the growth of the test organism, as demonstrated by the consistently higher mean zone of inhibition across all time intervals. Table no. 2 overall inter group comparison of all the 5 groups at 3 different time intervals using Anova. The mean zone of inhibition for the zinc oxide (ZOE) group was highest at 0 hours (11.75 mm), followed by a decrease at 7 days (8.25 mm) and a further decrease at 29 days (6.25 mm) for he the zinc oxide + clindamycin gel group, the mean zone of inhibition was highest at 0 hours (13.50 mm), followed by a decrease at 7 days (9.00 mm) and a further decrease at 29 days (6.50 mm).in the endoflas group, the mean zone of inhibition was consistent

Table 1. Intra group comparison of all the 4 groups at 3 different time intervals using Anova

Groups	Number of re-tests done	Mean zone of inhibition at 0 hours in mm	Mean zone of inhibition at 7th day in mm	Mean zone of inhibition at 30th day in mm	P value
Zinc oxide	11	11.75±2.36	8.25±0.95	6.25±0.50	0.00182
Zinc oxide + clindamycin gel	11	13.50±1.73	9.0± 2.70	6.5± 1.73	0.00349
Endoflas	11	11.75± 1.50	9.25± 2.06	7.00 ±1.63	0.0126
Endoflas+ clindamycin gel	11	13.25± 2.06	12.00± 0.81	8.00 ±0.81	0.00105

P<0.05 was considered as statistically significant.

Table 2. Overall inter group comparison of all the 5 groups at 3 different time intervals using Anova

Time intervals	Number of re-tests done	Zoe	Zinc oxide + clindamycin gel	Endoflas	Clindamycin gel + endoflas	P – value
0 hours	11	11.75±2.36	13.50±1.73	11.75 ±1.50	13.25± 2.06	0.0195
7 days	11	8.25± 0.95	9.00± 2.70	9.25 ±2.06	12.00 ±0.81	0.00478
29 days	11	6.25 0.50	6.50 ± 1.73	7.00 ± 1.63	8.00 ± 0.81	0.00884

P<0.05 was considered as statistically significant.

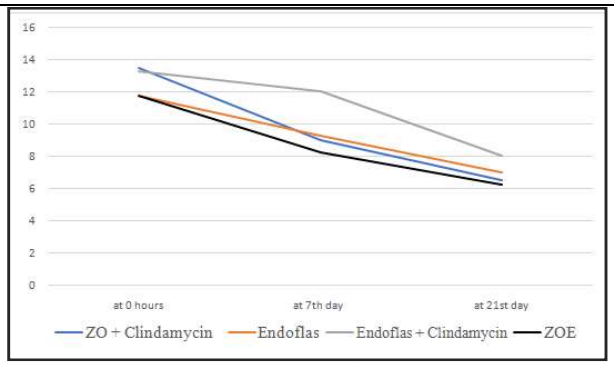

Table 3. Zone of inhibition(in mm) by various study groups at 3 different time points

Figure 1 . Access opening under rubber dam

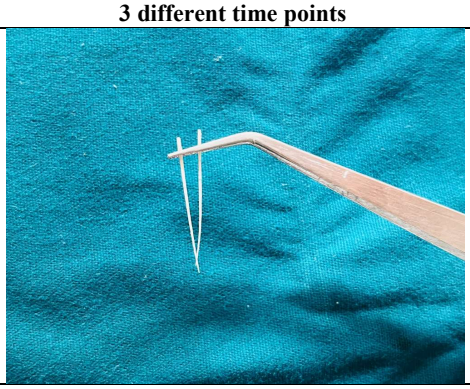

Figure 2. Microbial samples collected using a sterile paper point

Figure 3. Paper points sorted in cryovial with peptone water

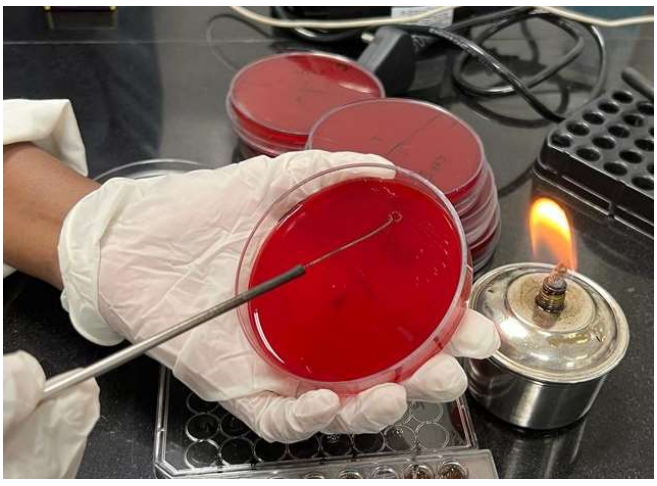

Figure 4 . Bacterial inoculated on blood agar plates

Figure 5. Zone of bacterial growth inhibition on the surrounding each obturating material

at 0 hours (11.75 mm) and 7 days (9.25 mm) but decreased slightly by 29 days (7.00 mm). The clindamycin gel + Endoflas group had a mean zone of inhibition of 13.25 mm at 0 hours, 12.00 mm at 7 days, and 8.00 mm at 29 days. Statistical analysis showed significant differences ($p < 0.05$) in the mean zone of inhibition between the groups at each time interval, indicating variations in the effectiveness of the treatments over time.

DISCUSSION

Thin, tortuous and complex nature of the root canal system and change in their morphology with root resorption makes it difficult to achieve proper disinfection by mechanical instrumentation and irrigation of the canals.[1] A part of the root canal space often remains untouched during chemo mechanical preparation regardless of the technique and instruments employed. Studies reported the presence of microorganisms in areas such as isthmus, ramifications, deltas, irregularities, and dentinal tubules even after thorough chemo mechanical debridement of the root canal system. [10] The risk of treatment failure increases if microorganisms grow and colonize in the dentine tubules despite irrigation of the root canal.[11] The prevalence of *enterococcus faecalis* bacteria in root canal treatment failure ranges from 24% to 77%.[12] as the *enterococcus faecalis* bacteria can withstand extreme environments and very alkaline hydrogen (pH) potential, and high salt concentrations.[13] Use of right intracanal medicament material is needed to reduce the number or kill root canal bacteria to prevent reinfection.[14] Hence, obtaining a hermetic seal of the root canals with a root canal filling material that possesses an excellent antimicrobial property is critical for endodontic success. Bacterial re-entry and growth of residual micro-organism may be prevented by incorporation of antimicrobial components into obturating materials.[15] Thus, the present study was conducted to evaluate if there is an enhanced antimicrobial activity when a broad-spectrum antimicrobial agent namely clindamycin gel was incorporated into zinc oxide eugenol and Endoflas FS. It is advised to test the dental materials immediately after mixing, once the final chemical setting stage has been reached. This is because of the formation of various temporary or permanent by-products during the setting reaction which may influence the original results. Thus in the present study, the freshly prepared root canal filling materials were placed into agar plates. The agar diffusion method was used in our study to evaluate the antibacterial efficacy as it has been widely used to test the antimicrobial activity of dental materials and medicaments.[16]

However, this procedure is influenced by two factors: the material's microbial toxicity as well as the materials affinity and diffusibility in the culture medium. A material that easily diffuses will produce larger zones of inhibition of bacteria. The results of the present study showed Endoflas FS has better antimicrobial properties when compared with zinc oxide eugenol. The antimicrobial effect of eugenol based obturating material was mainly attributed to the action of eugenol. Eugenol acts on micro-organism by causing protein denaturation rendering it non-functional. The results of the various studies performed by Gomes *et al*., Markowitz *et al*., and Sagar *et al*., also confirmed that eugenol containing obturating materials were more superior in inhibiting the microorganisms. [17,18,19] Endoflas FS showed Better antimicrobial activity than ZOE obturating material probably due to incorporation of known bactericidal agents such as iodoform. Iodoform acts by the liberation of iodine. It is believed that iodine, which is an oxidizing agent, can irreversibly oxidize and thus, inactivate essential metabolic compounds like proteins, nucleotides and fatty acid resulting in cell death, but the exact mode of action is not fully known. Similar results have been reported by Gopikrishna *et al*., and Kayaoglu *et al*., [20,21] Disadvantage of causing tooth discoloration and periapical irritation due to eugenol component.[22] Studies reported that iodoform based root canal filling materials show cytotoxic effects and eugenol component present in the Endoflas show hypersensitivity or allergic reactions when it comes in contact with soft tissue.[23] In order to overcome some of these limitations with Endoflas, clindamycin gel was tried as an alternative to liquid component eugenol as obturating material for primary teeth. Clindamycin is proven to have broad-spectrum antimicrobial activity and substantivity. Efficient in the treatment of acute infection and flare-ups and abscesses. The antibiotic can fight various anaerobic, facultative, and anaerobic bacteria.[24] Clindamycin is effective against various endodontic pathogens in root canals and dentinal tubules.[25] The mechanism of action of clindamycin hydrochloride is to inhibit peptide bonds formed from bacterial deoxyribonucleic acid (DNA) that cause cell death.[26] The decrease in the number of bacteria is possible as clindamycin is bacteriostatic, which inhibits bacterial protein.[27] It is in line with the study by Sunder *et al*., which states that clindamycin was effective against enterococcus faecalis after 24 hours of incubation in agar media.[28] In the present study, the antimicrobial activity of all the tested root canal filling materials gradually decreased with time, the highest being at the end of 24 hours and the lowest being at the end of the 29th day. Based on the results of the present study, we recommend the use of 2% clindamycin gel with Zinc oxide

eugenol or Endoflas as root canal filling materials in highly infected primary teeth requiring pulpectomy to improve the success of the endodontic therapy. Further studies evaluating the same under *in vivo* conditions may help in substantiating the obtained results of this study.

CONCLUSION

Under the limitations of this in vitro study, Clindamycin gel significantly enhanced the antimicrobial activity of Zinc oxide eugenol and Endoflas against E. faecalis with long lasting antibacterial effect up until day 30. Hence**,** further in-vivo studies with larger sample size are needed to evaluate the antimicrobial efficacy of root canal obturating materials, in clinical settings.

Glossary of Abbreviations

- E. *faecalis* – Enterococcus faecalis
- ZOE – Zinc oxide eugenol

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