

Study Protocol

Evaluation of a Triple Antibiotic Paste as a Root Canal Obturating Material for Deciduous Teeth: A Study Protocol

ABSTRACT

Background: Teeth with infected root canals, secondary to endodontic infections are a common problem in primary as well as permanent dentition. The key to successful endodontic treatment is seen only after the complete removal of infection from root canals. Systemic antibiotics have limited reach to endodontic spaces. In pediatric dentistry, difficulties in antimicrobial control require the development of a new root canal obturating paste with broad antimicrobial activity, minimal tissue toxicity, and high safety index. In this experimental study, a new Triple Antibiotic Obturating Paste (TAOP) will be developed and its efficacy will be evaluated.

Method and Design: This is a pre-clinical in-vitro microbiological and in-vivo animal study, utilizing laboratory-grown white Wistar rats aged 2-3 months and weighing 200-300 grams. The antibiotic sensitivity test (AST) will be conducted using 21 standard aerobic and anaerobic microbial strains for determining potency through serial dilution and agar diffusion assay. The systemic and local tissue toxicity will be assessed by inserting the test and control materials into the dorsal connective tissues of the experimental rats. The antimicrobial efficacy of triple antibiotic obturating paste [thermo-modulated in-situ polymeric hydrogel matrix of TAOP, containing chitosan-carbopol and poloxamer blended with clindamycin (5%), metronidazole (5%), and doxycycline (1%)] will be evaluated against Metapex [Ca(OH)₂-Iodoform paste] on endodontic microflora. The microbiological study data will be evaluated through One-way ANOVA trailed by 'Tuckey's post hoc test' for intergroup as well as, t-test for intragroup comparison. The animal study data will be evaluated through One-way ANOVA for intergroup and 'paired t-test' for intragroup comparison.

Results: The newly developed bio-degradable obturating material will be more suitable than the conventionally used one, to eliminate resistant endodontic pathogens from root canal systems, along with good pharmacokinetic and pharmacodynamic responses as well as minimal local and systematic tissue toxicity when used in primary teeth.

Conclusion: The application of such novel multi-antibiotic formulation as an obturating material not only will achieve efficient disinfection of the endodontic spaces but also increase chances of therapeutic success due to the elimination of side effects associated with cytotoxicity and material properties as it contains most of the bioresorbable organic ingredients.

Keywords: Antimicrobial agents; Clindamycin; Deciduous teeth; Doxycycline; Metronidazole; Obturating Material; Root Canal; Triple Antibiotic Paste.

Background:

Teeth with infected root canals, secondary to endodontic infections are a common problem in primary as well as permanent dentition.¹ Cultivable microbial isolated from such root canals have shown the presence of pure aerobic, anaerobic as well as mixed ecology of gram-positive and negative cocci, bacilli, and fungi.^{2,3,4} Complete removal of causative

agents is key to successful endodontic treatment⁵, including procedures like pulpotomy, pulpectomy, or root canal therapy to preserve teeth in dental arches successfully.¹ Such therapy help remove microorganisms from root canals along with their by-products employing chemical and mechanical cleansing.⁶ The disinfected root canals are filled with various obturating materials like zinc-oxide eugenol (ZOE) and its various combinations, vitapex/metapex [Ca(OH)₂-Iodoform paste], Guedes-Pinto paste, KRI paste, and Maisto paste.⁶ Though different non-specific antibacterial and antibiotic intracanal medicaments used in pediatric endodontics suppress pathogenic microbes initially but fail to prevent repopulation of the residual one later.⁷ Thus, difficulties in microbial control necessitate the use of root canal obturating pastes with broad antimicrobial activity.

Systemic antibiotics are usually advocated to alleviate symptoms associated with pulp space and/or periapical area infections.⁷ These agents are considered as an adjunct to definitive endodontic therapy. For a systemic antibiotic to effectively eliminate bacteria, has to reach pulp space through blood supply which is usually compromised in infected and necrotic pulp limiting its penetration and efficacy.⁷ Thus, along with empirical systemic antibiotics, topical application of antibiotic combination emerged to target endodontic pathogens and prevent resistance, giving rise to “the concept of lesion sterilization and tissue repair (LSTR)”. Though the results obtained through this procedure were promising^{5,7,8}, it imposed few untoward results like tissue degeneration and necrosis⁹, incompatibility with periradicular tissues¹⁰, and discoloration of teeth.¹¹ The drug concentrations used in previous ‘LSTR studies’ (like; 3 parts ciprofloxacin, 3 parts metronidazole, and 1 part minocycline) were also very high causing damages.¹² The antibacterial drugs used in previous studies were ciprofloxacin, minocycline, and metronidazole.

Ciprofloxacin is a broad-spectrum antimicrobial agent with a high bactericidal effect acting through attachment at two of the four bacterial topoisomerases.^{13,14} However it has been reported with adverse drug reactions like aberrant hepatic function tests, altered total and differential leucocytic counts, nausea and, vomiting in adults.¹⁵ In pediatric patients, the use of ciprofloxacin and fluoroquinolones has been warranted due to various musculoskeletal adverse effects like arthropathy in weight-bearing joints, mostly the knee joints due to cartilage damage caused by quinolones.¹⁶ Minocycline is a bacteriostatic antibiotic that helps to eradicate bacteria from the root canals but has the disadvantage of causing tooth discoloration. Minocycline when comes in direct contact with the tooth surface gets attached to a glycoprotein of the acquired pellicle.¹⁷ This complex alters the enamel surface due to etching due to exposure to air or as an outcome of bacterial activities producing oxides and transmuting non-soluble dark tenacious blackish quinone deposits.^{18,19} Hemosiderin and minocycline degradation products in the form of iron chelates lead to the formation of insoluble complexes.¹⁸ As the problems associated with the previously used materials steel persist, a search for an ideal obturating material for primary teeth continues.

Whenever a new drug is developed, the determination of proper drug dosage is essential to eliminate problems associated with the safety index. Especially in pediatric dentistry, difficulties in antimicrobial control require the development of a new root canal obturating paste with broad antimicrobial activity; minimal tissue toxicity, and high safety index. Thus, in this experimental study, a new Triple Antibiotic Obturating Paste (TOAP) will be developed for primary teeth that should meet the criteria of an ideal obturating material for primary teeth and achieve better “lesion sterilization and tissue repair (LSTR)”.

RESEARCH QUESTION: Can the formulation of Triple Antibiotic obturating Paste (TAOP) be proven as a better obturating material for primary teeth in terms of antimicrobial efficacy, local and systemic toxicity?

HYPOTHESIS: Formulation of Triple Antibiotic obturating Paste (TAOP) can be proven to be better obturating material for primary teeth in terms of eliminating endodontic pathogens effectively from root canal systems as well as reducing local & systemic toxicity, through microbiological and animal study and achieving better “Lesion Sterilization and Tissue Repair (LSTR)”.

NULL HYPOTHESIS: Formulation of Triple Antibiotic obturating Paste (TAOP) for “lesion sterilization and tissue repair (LSTR)” is not dependent on in-vivo antibacterial efficacy as well as local & systemic toxicity for the complete elimination of endodontic pathogens from root canal systems of primary teeth.

AIM AND OBJECTIVES OF THE STUDY:

Aim of the study: To evaluate the efficiency of newly formulated Triple Antibiotic obturating Paste (TAOP) for primary teeth.

Objectives of the study:

- 1) Determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the Triple Antibiotic obturating paste (TAOP) against endodontic microflora.
- 2) Compare and evaluate the antimicrobial efficacy of Metapex [$\text{Ca}(\text{OH})_2$ -Iodoform paste] and the Triple Antibiotic obturating Paste (TAOP) on endodontic microflora.
- 3) Compare and evaluate the systemic and local toxicity of Metapex [$\text{Ca}(\text{OH})_2$ -Iodoform paste] and the Triple Antibiotic obturating Paste (TAOP) – in albino rats.

MATERIAL AND METHODS:

Study setting: The study will be conducted at the Department of Pedodontics and Preventive dentistry, SPDC; and the Department of Pharmacology JNMC, with an association of Department of Microbiology, Department of Pathology, JNMC; and Department of Oral Pathology and Microbiology, SPDC; DMIMS Sawangi.

Ethical Committee Approvals: The protocol for the present in-vitro study has been approved by Institutional Ethical Committee, letter number DMIMS (DU)/IEC/2015-16/1744, dated December 31, 2015, while for in-vivo animal study by the Institutional Animal Ethical Committee Board letter-number DMIMS (DU)/IAEC/2015-16/1, following the guidelines of “CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals)”.

Study Design: This is a pre-clinical in-vitro microbiological and in-vivo animal study.

Source of data:

For Microbiological study - The microbiological research work will be conducted at the Department of Pedodontics, SPDC, and Department of Microbiology, JNMC Sawangi (M), after approval of the ethical committee. The samples used in this study will be endodontic micro-organisms and non-pathogenic pure culture of ATCC / MCC type micro-organisms.

For Animal study - An experimental animal study will be conducted on albino rats. [Animal Ethics Committee Permission – The study will be initiated only after obtaining permission from the Animal Ethics Committee of the Institution.]

Participants:

For microbiological study - for microbiological study, two different types of microorganisms will be used.

1) ATCC / MCC type non-pathogenic endodontic micro-organisms will be procured from microbiological labs.

2) Endodontic microbes will be collected from infected primary teeth of 4 – 10 years children randomly from OPD of the Department of Pedodontics.

For animal study - A total of 48 albino rats weighing 200 – 300 grams will be selected from animal-house maintained by the DMIMS (DU).

Sampling procedure:

For microbiological study - Samples used in microbiological study should meet the following inclusion and exclusion criteria.^{2-4,20,21}

Clinical Inclusion criteria:

- 1] Extensive dentinal caries lesions showing pulp exposure, where bleeding can't be arrested after coronal pulp amputation, spontaneous pain, pain on percussion or abnormal mobility,
- 2] Clinical examination revealing acute or chronic periapical abscess, sinus tract or fistula or periapical pathology due to dental caries with the associated tooth,
- 3] Tooth containing infected or necrotic pulp in at least one root canal.

Radiographic Inclusion Criteria:

- 1] Evidence of deep carious lesion showing coronal pulp encroachment.
- 2] Radiographic evidence of furcation involvement without any type of root resorption.
- 3] Extensive radicular and furcation area bone loss not evading the underlying tooth germ.
- 4] Radiographic appearances of root and supportive structure that can be categorized into any of the following 4 groups:

Chart 1. Radiographic appearances of root and supportive structure

Score	Radiographic findings
P1	No pathology
P2	Discontinuity of lamina dura
P3	Furcation involvement \leq half of the shortest root of deciduous posterior teeth in vertical measurement
P4	Furcation involving more than half of the shortest root

Clinical Exclusion criteria:

- 1) **Patients** who have received antibiotic therapy 4 weeks before sampling procedure.

2) Teeth showing perforated pulpal floor or gross carious destruction that cannot be restored clinically.

Radiographical Exclusion Criteria:

- 1) Radiographic indication of excessive internal/ external root resorption.
- 2) Radiographic evidence of pulpal floor perforation and/or gross destruction of the affected tooth.

For animal study - A total of 48 albino rats weighing 200 – 300 grams will be selected randomly from the animal house. All the animals will be divided into 03 equal groups, each group having 16 animals.

Materials required for the study:

Chart 2. For microbiological study - Test material and Negative and Positive control groups will be used,

Group	Test Material
Group A (Negative Control)	Treated with sterile Petroleum jelly
Group B (Positive Control)	Metapex [$\text{Ca}(\text{OH})_2$ -Iodoform paste]
Group C (Test Material)	Triple Antibiotic obturating Paste (TAOP)

Chart 3. For animal study - 1 Test material and a Negative and Positive Control group will be used.

Group	Treatment
Group A Negative Control	No treatment done.
Group B Positive Control	Metapex [$\text{Ca}(\text{OH})_2$ -Iodoform paste]
Group C Test Material	Triple Antibiotic obturating Paste (TAOP)

Sample size for In-vitro microbiological study: For the power of the probability 90%, for finding a statistically significant difference at a given $p = 0.01$ with a specified sample, $n = 21$ micro-organisms (types) of ATCC / MCC type and organisms isolated from root canals of patients in each group will be taken.

$$n = \log \beta / \log p$$

β is the probability of committing Type II Error (Usually 0.10 or 0.05)

p represents proportions of micro-organisms that are unaffected

In the microbiological study - 21 standard aerobic and anaerobic microbial strains obtained from ATCC / MCC laboratories (non-pathogenic standard endodontic microbial strains), as well as pathogenic aerobic and anaerobic microbial strains isolated from endodontic specimens of patients, will be used.

Sample size determination for the animal study²²: For determining sample size in the animal study, two methods for calculation of sample size can be employed. The most favored one is by ‘the hypothesis testing’. But, occasionally, the above-mentioned method doesn’t give sufficient evidence for sample size calculation through various power analyses like standard deviation, effect size, etc. Hence, another method is known as the “Resource Equation Method” can be used alternatively. In this method, a pre-decided sample size can be used to calculate value “E”, which should lie within 40 to 50 for optimal experimental sample size. If the value of “E” is less than 40, higher study samples (experimental animals) should be included, and if it is more than 50, then they should be reduced.

$$* E = \text{Total number of animals} - \text{Total number of groups}$$

Thus, in the present experimental study, 03 sample groups each having 16 animals, for different tests will be formed, thus the total number of animals will be $(03 \times 16) = 48$. Hence “E” will be -

$$E = 48 - 3 = 45$$

In this study E is 48; the value of E is within the optimum level of the “Resource Equation Method”. Hence there is no need to increase or decrease the sample size. If the researcher takes approximately 16 rats in each group for 03 groups then it can be considered as an appropriate sample size.

Chart 4. Grouping of Animals: 48 adult albino rats aged 2-3 months and weighing 200 to 300 grams will be divided into 03 groups of 16 animals each by random allocation.

Groups with specifications of Treatment (N=16 and, n=8)		
Groups		Treatment modality
Group A	Negative Control	No treatment
Group B	Positive control	Metapex [Ca (OH) ₂ -Iodoform paste]
Group C	Test group	Triple Antibiotic obturating Paste (TAOP).

Chart 5. Each group will be further subdivided into 2 subgroups (n=8) as –

Treatment group	Toxicity evaluation	Duration
Group A (Negative Control)	Systemic and local	15 days
	Systemic and local toxicity	3 months
Group B (Positive Control)	Systemic and local toxicity	15 days
	Systemic and local toxicity	3 months
Group C (Test group)	Systemic and local toxicity	15 days
	Systemic and local toxicity	3 months

Methodology for microbiological study: The microbiological study will be carried out in two stages. In the first stage, 21 standard aerobic and anaerobic microbial strains obtained from ATCC / MCC laboratories (non-pathogenic standard endodontic microbial strains) will be used for determining potency, antibiotic activity (AST), and Minimum Inhibitory Concentration (MIC) as well as standardization of all test materials by agar diffusion assay and serial dilution method as well as standardization of test materials dosage.^{23,24}

In the second stage, the endodontically involved teeth will be isolated with a rubber dam and under all aseptic conditions, the microbial samples will be collected from the root canals of the affected tooth. The micro-organisms will be isolated and kept in culture media for growth in microbiology laboratory under strict aseptic conditions.^{2,6} Samples will be then transported to the Department of Microbiology for aerobic and anaerobic culture in nutrient broth and Robertson's cooked meat (RCM) medium respectively. After 2 hours of incubation, the aerobic subculture will be streaked on 5% blood agar and MacConkey's agar and kept for incubation at 37°C in an atmosphere containing 5% CO₂ for 48 hrs. From RCM medium, subculture will be done after 48 hrs on 5% Blood Agar and incubated anaerobically. Then the aerobic and anaerobic isolates will be identified based on their morphology in gram stain smear, colony characteristic, and specification will be done by standard biochemical test. The Antibiotic activity of all the test materials will be tested against these isolates by agar diffusion assay and serial dilution method.^{2,3}

Methodology for animal study: The animals used in this study will be kept at a temperature-controlled animal house and fed with food and water ad-libitum. The animals will be housed and taken care of as per the guidelines given by the "Animal Ethical Committee" and "Committee For the Purpose of Control and Supervision on Experiments on Animals (CPCSEA)" after approval.

Total sixty-four (64) sterile, ten millimeters long, polyethylene terephthalate (PET) tubes with an internal diameter of 1.1 mm and, 1.6 mm external diameter will be used in this study. In each animal, two tubes will be inserted, one empty tube and one tube including test material.^{25,26} In the negative control group no treatment will be done on animals. They will be used as a reference against test groups. In the positive control group, empty tubes and tubes filled with Metapex [Ca (OH)₂-Iodoform paste] will be inserted. In the test material group, empty tubes and tubes filled with Triple Antibiotic obturating Paste (TAOP) will be inserted.^{27,28}

For insertion of experimental material in animals following procedure will be followed. The animals will be kept for fasting overnight. The next day the animal will be anesthetized using Ketamine HCl (22-24 mg/kg) by IM route.²⁹ Under all the aseptic conditions throughout the experiment, the dorsal skin of all the animals will be shaved and disinfected with 5% povidone-iodine solution. A craniocaudal incision, approximately 2 cm, will be given on the shaved dorsal skin of animals with the help of a BP blade (15 no. Bard-Parker blade). Two subcutaneous pockets will be created by blunt tissue dissection and skin reflection, six centimeters apart from each other, on each side of the incision. Sterile PET tubes filled with test materials, as described previously will be implanted in the subcutaneous tissue space created by blunt dissection and the skin will be sutured back using 4-0 chromic catgut suture.^{30,31} Suitable analgesic will be given to animals to reduce their post-operative pain and increase the survival rate of experimental animals.²⁹ Animals will be observed in the animal house for the stipulated period carefully.

Chart 6. Animals will be observed for 2 types of evaluations –

Type of animal evaluations	Duration	Mode of evaluation
systemic toxicity of the drugs	at 15 days and 3 months interval	clinical and histological evaluation
local toxicity of the drug	at 15 th day and 3 months interval	clinical and histological evaluation

Evaluation of animals for systemic and local toxicity of drugs: All the animals of subgroups A will be euthanized on the 15th day and subgroup B will be euthanized at 3-month intervals with an overdose of ketamine²⁹ to retrieve their livers, kidneys, brains, and tubes with surrounding connective tissues. The retrieved tissue samples will be treated with 10% buffered neutral formalin at pH 7.0, for comparative evaluation of the systemic and local toxicity of all the test materials through histopathological examination.²⁸ The retrieved tissue samples will be carried to the Department of Pathology, for further histopathological evaluation and analysis.

Criteria for systemic toxicity evaluation of drug – Samples of livers, kidneys, and brains will be collected after sacrificing the animals, by excisional biopsy on the 15th day and 3 months interval. The samples will be fixed in 10 % neutral buffered Formalin. The histopathological evaluation will be performed on hematoxylin and eosin-stained paraffin-mounted thin sections of tissues for degenerative or necrotic changes in tissues against normal tissues of untreated animals.

Criteria for local toxicity evaluation of drug – Areas of experimental animal dorsal connective tissue with polyethylene tubes will be removed at the 15th day and 3-month interval. The samples will be fixed in 10 % neutral buffered Formalin. The tubes will be divided into two transverse halves, longitudinally with the help of a sharp blade allowing contact of all the issue surfaces to maintain contact with processing solutions. The specimens will be paraffin-embedded and sectioned serially to stain with hematoxylin and eosin (H & E stain).²⁸

Chart 7. The reactions of the animal tissues coming in contact with test materials at the tube openings will be scored against tissue around empty tubes without material as follows:^{23,29}

Score	Criteria
0	No or few inflammatory cells and no reaction
1	Less than 25 cells and mild reaction
2	Between 25 and 125 cells and moderate reaction
3	125 or more cells and severe reaction

The fibrous capsule surrounding polyethylene tubes received from experimental animals will be classified as ‘thin’ if the thickness of the capsule is < 150 um or ‘thick’ if the thickness is > 150 um. Necrosis and calcifications in the histological specimens will be recorded as present or absent. An average number of cells in each group will be obtained from scoring 10 separate areas. Local toxicity changes within tissues will also be recorded for the following aspects: Inflammatory response and degeneration/necrosis of connective tissue

cells.^{34,33,35} All the observers, familiar with the histopathological evaluations will be blinded from the treatment to avoid inter-observer bias.

Data management and Statistical Analysis plan: All the data collected will be tabulated and analyzed as follows. For the In-vitro microbiological study, all the groups will be analyzed by One-way ANOVA (ANALYSIS OF VARIANCE) followed by ‘post-hoc Tukey's test’, Dunnett D for group-wise comparison, and ‘t-test’ for the effectiveness of drugs. For the In-vivo animal study, the results obtained from the preclinical study will be analyzed using One-way ANOVA, Student's paired ‘t-test’.

Expected Outcome: The newly developed bio-degradable obturating material will be **more suitable** than the conventionally used one, to eliminate resistant endodontic pathogens from root canal systems, along with good pharmacokinetic and pharmacodynamic responses as well as minimal local and systematic tissue toxicity when used in primary teeth.

Scope of the study: The proposed preclinical study has the following scopes:

- 1) To develop a suitable bio-degradable obturating material for primary teeth that will be able to eliminate resistant endodontic pathogens from root canal systems.
- 2) To evaluate pharmacokinetic and pharmacodynamic responses of the newly formulated drug.
- 3) Results obtained in this study will be helpful for further clinical trials in terms of safety index in the future.

Limitation of the study:

- 1) Triple Antibiotic obturating Paste (TAOP) will not be able to be used in patients having allergic reactions to its components.
- 2) In the microbiological study, local and systemic effects on living tissues (healthy and/or diseased) in terms of pharmacokinetics and pharmacodynamics of developed drug **combinations** cannot be judged adequately.
- 3) As this is a preclinical study, local and systemic effects documented in living tissues of animals may differ from human tissue response that necessitates controlled In-vivo clinical trials in human volunteers in the future.

Clinical Implications:

- 1) Triple Antibiotic obturating Paste (TAOP) can be used effectively in all endodontic infections to achieve root canal disinfection.
- 2) It can be used in endo-perio lesions to achieve the elimination of pathogenic bacteria from the periodontal pocket and help in bone and PDL regeneration.
- 3) This study will open new horizons of endodontic treatment modalities for primary as well as permanent teeth in terms of regenerative endodontics.
- 4) In specific conditions like calcified/obliterated root canals, primary teeth with premature pathological resorption having strategic importance in space management in the dental arch, un-cooperative children where instrumentation of root canals is difficult, this material can be used to achieve Lesion Sterilization and Tissue Repair (LSTR) preserving teeth in the oral cavity in a healthy functional state.

DISCUSSION:

Endodontic pathogens acquired from the oral cavity, carious tooth, anachoresis, or pre-contaminated inadequately sterilized dentinal tubules, can be either facultative or obligate, aerobes, and anaerobes.³⁶ The degradation products from damaged pulp provide

favorable environments for multi-microbial colonization and growth inside pulp space. Few of them form biofilms along the tortuous inaccessible spaces retaining microbial remnants after exhaustive chemo-mechanical preparation and irrigation³⁷, regardless of the irrigation systems, leading to therapeutic failures and uncertain prognoses.³⁸ Although obturating materials with antibacterial properties eradicate such residual remnants, essentially in teeth with apical periodontitis, pulp necrosis, and refractory endodontic conditions, disburdening systemic antimicrobial therapy⁷, they are inefficient to eliminate many microbial species.³⁹ Likewise, such materials need to be minimally cytotoxic and biologically safe for tissues in close vicinity to them.⁴⁰ Zinc oxide eugenol (ZOE)⁴¹ and other materials like calcium hydroxide iodoform paste or its modifications (e.g. Vitapex, Metapex, and KRI paste)^{42,43}, induce strong chronic inflammatory reactions in the tissues they contact, impedes cell functions and, hinder physiological root resorption due to poor phagocytosis. Zinc oxide and eugenol (ZOE) alter the eruption path of the permanent tooth.⁴⁴ Calcium hydroxide-based obturating materials, despite acceptable biological properties⁴⁵, show high solubility in tissue fluids and within the root canals.⁴⁶ To overcome such flaws, the use of antimicrobial agents individually, or in combination with other nonantibiotic agents, like double antibiotic paste (DAP: metronidazole and ciprofloxacin), modified triple antibiotic paste (MTAP: minocycline component replaced), gained popularity to use in form of obturating materials to preserve primary and young permanent teeth in uncooperative children.⁴⁷ Besides reduction in the systemic toxicity, application of multi-antimicrobial preparations has shown to achieve nearly complete microbial elimination at very low concentrations.⁴⁸ However, the exact doses of such agents were undetermined, and the concerns regarding systemic adverse effects due to prolonged exposure of individual agents were also not addressed previously.⁴⁹

Though optimum antimicrobial and minimal cytotoxicity are desirable properties of an obturating material⁵⁰, accidental plunging of such agents into the periapical region evokes immunological responses. The reports from previous experiments revealed high cytotoxicity of ZOE and metapex.⁵¹ The disadvantages of these materials encouraged the invention of new antibiotic-containing materials to minimize aforesaid properties through planned in-vitro as well as in-vivo experimental setups. With the lack of evidence for such information, an attempt was made in this experiment to develop a precisely calibrated, minimally cytotoxic, and optimally antimicrobial multi-antibiotic combination in a user-friendly form of delivery.⁵³⁻⁵⁴

CONCLUSION:

The application of such novel multi-antibiotic formulation as an obturating material not only will achieve efficient disinfection of the endodontic spaces but also increase chances of therapeutic success due to the elimination of side effects associated with cytotoxicity and material properties as it contains most of the bioresorbable organic ingredients.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research will be commonly and predominantly used in our area of research and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

REFERENCES:

1. Fuks AB. Pulp therapy for the primary and young permanent dentitions. *Dent Clin North Am.* 2000;44(3):571-596, vii.
2. Rani A, Chopra A. Isolation and identification of root canal bacteria from symptomatic nonvital teeth with periapical pathosis. *Endodontology.* 2006;18(1):12-17.
3. Ercan E, Dalli M, Yavuz İ, Özekinci T. Investigation of Microorganisms in Infected Dental Root Canals. *Biotechnol Biotechnol Equip.* 2006;20(2):166-172. doi:10.1080/13102818.2006.10817361
4. Ferrari PHP, Cai S, Bombana AC. Effect of endodontic procedures on enterococci, enteric bacteria and yeasts in primary endodontic infections. *Int Endod J.* 2005;38(6):372-380. doi:10.1111/j.1365-2591.2005.00947.x
5. Sato I, Ando-Kurihara N, Kota K, Iwaku M, Hoshino E. Sterilization of infected root-canal dentine by topical application of a mixture of ciprofloxacin, metronidazole and minocycline in situ. *Int Endod J.* 1996;29(2):118-124. doi:10.1111/j.1365-2591.1996.tb01172.x
6. Amorim L de FG de, Toledo OA de, Estrela CR de A, Decurcio D de A, Estrela C. Antimicrobial analysis of different root canal filling pastes used in pediatric dentistry by two experimental methods. *Braz Dent J.* 2006;17(4):317-322. doi:10.1590/s0103-64402006000400010
7. Mohammadi Z, Abbott PV. On the local applications of antibiotics and antibiotic-based agents in endodontics and dental traumatology. *Int Endod J.* 2009;42(7):555-567. doi:10.1111/j.1365-2591.2009.01564.x
8. Takushige T, Cruz EV, Asgor Moral A, Hoshino E. Endodontic treatment of primary teeth using a combination of antibacterial drugs. *Int Endod J.* 2004;37(2):132-138. doi:10.1111/j.0143-2885.2004.00771.x
9. Wang X, Thibodeau B, Trope M, Lin LM, Huang GT-J. Histologic Characterization of Regenerated Tissues in Canal Space after the Revitalization/Revascularization Procedure of Immature Dog Teeth with Apical Periodontitis. *J Endod.* 2010;36(1):56-63. doi:10.1016/j.joen.2009.09.039
10. Yoshida K, Yoshida N, Iwaku M. Effects of antibacterial capping agents on dental pulps of monkeys mechanically exposed to oral microflora. *J Endod.* 1995;21(1):16-20. doi:10.1016/S0099-2399(06)80551-0
11. Akcay M, Arslan H, Yasa B, Kavruk F, Yasa E. Spectrophotometric Analysis of Crown Discoloration Induced by Various Antibiotic Pastes Used in Revascularization. *J Endod.* 2014;40(6):845-848. doi:10.1016/j.joen.2013.09.019
12. Labban N, Yassen GH, Windsor LJ, Platt JA. The direct cytotoxic effects of medicaments used in endodontic regeneration on human dental pulp cells. *Dent Traumatol.* 2014;30(6):429-434. doi:10.1111/edt.12108

13. Adefurin A, Sammons H, Jacqz-Aigrain E, Choonara I. Ciprofloxacin safety in paediatrics: a systematic review. *Arch Dis Child*. 2011;96(9):874-880. doi:10.1136/ad.2010.208843
14. Gendrel D, Chalumeau M, Moulin F, Raymond J. Fluoroquinolones in paediatrics: a risk for the patient or for the community? *Lancet Infect Dis*. 2003;3(9):537-546. doi:10.1016/S1473-3099(03)00736-9
15. Ball P. Ciprofloxacin: an overview of adverse experiences. *J Antimicrob Chemother*. 1986;18(Supplement_D):187-193. doi:10.1093/jac/18.SD.187
16. Ingham B, Brentnall DW, Dale EA, McFadzean JA. Arthropathy induced by antibacterial fused N-alkyl-4-pyridone-3-carboxylic acids. *Toxicol Lett*. 1977;1(1):21-26. doi:10.1016/0378-4274(77)90016-9
17. Ayaslioglu E, Erkek E, Oba A, Cebecioglu E. Doxycycline-induced staining of permanent adult dentition. *Aust Dent J*. 2005;50(4):273-275. doi:10.1111/j.1834-7819.2005.tb00373.x
18. Good ML, Hussey DL. Minocycline: stain devil? *Br J Dermatol*. 2003;149(2):237-239. doi:10.1046/j.1365-2133.2003.05497.x
19. Patel K, Cheshire D, Vance A. Oral and systemic effects of prolonged minocycline therapy. *Br Dent J*. 1998;185(11):560-562. doi:10.1038/sj.bdj.4809867
20. Davis JM. Endodontic therapy in the primary dentition. *Dent Clin North Am*. 1979;23(4):663-672.
21. Trairatvorakul C, Chunlasikaiwan S. Success of pulpectomy with zinc oxide-eugenol vs calcium hydroxide/iodoform paste in primary molars: a clinical study. *Pediatr Dent*. 2008;30(4):303-308.
22. Charan J, Kantharia ND. How to calculate sample size in animal studies? *J Pharmacol Pharmacother*. 2013;4(4):303-306. doi:10.4103/0976-500X.119726
23. Dahake PT, Baliga SM. Antimicrobial efficacy of a new tri-antibiotic combination against resistant endodontic pathogens: An in-vitro study. *Braz Dent Sci*. 2020;23(4):8p. doi:10.14295/bds.2021.v24i1.2186
24. Dahake PT, Jha RK, Baliga SM. Evaluation Of Combination Of Three Antimicrobial Agents Against Common Endodontic Pathogens. *Int J Sci Res*. 2019;8(11):63-64. doi:10.36106/ijsr
25. Gomes-Filho JE, Duarte PCT, de Oliveira CB, et al. Tissue Reaction to a Triantibiotic Paste Used for Endodontic Tissue Self-regeneration of Nonvital Immature Permanent Teeth. *J Endod*. 2012;38(1):91-94. doi:10.1016/j.joen.2011.09.020
26. Holland R, Gomes Filho JE, Cintra LTA, Queiroz ÍO de A, Estrela C. Factors affecting the periapical healing process of endodontically treated teeth. *J Appl Oral Sci*. 2017;25(5):465-476. doi:10.1590/1678-7757-2016-0464

27. Dahake PT, Baliga SM, Punse T, Biyani DM, Raut N, Umekar MJ. Formulation and Physical Characterization of Bio-Degradable Chitosan-Poloxamer Gel Base for Local Drug Delivery. *J Drug Deliv Ther.* 2020;10(4):59-66. doi:10.22270/jddt.v10i4.4216
28. Dahake PT, Baliga SM, Kumbar VM, Bhat KG. Cytotoxicity of Novel Polymeric Gel Matrix Triple Antibiotic Paste—an In Vitro Study. *Regen Eng Transl Med.* Published online December 2, 2020. doi:10.1007/s40883-020-00191-x
29. Majagi S, Karnad A, Patil P. Calcium enhances antiinflammatory activity of aspirin in albino rats. *Indian J Pharmacol.* 2006;38(6):397. doi:10.4103/0253-7613.28205
30. American National Standards Institute/American Dental Association Document no. 41 for Recommended Standard Practices for Biological Evaluation of Dental Materials. Council on Dental Materials and Devices. *J Am Dent Assoc* 1939. 1979;99(4):697-698.
31. Recommended standard practices for biological evaluation of dental materials. Fédération Dentaire Internationale, Commission of Dental Materials, Instruments, Equipment and Therapeutics. *Int Dent J.* 1980;30(2):140-188.
32. Karanth P, Manjunath MK, Roshni, Kuriakose ES. Reaction of rat subcutaneous tissue to mineral trioxide aggregate and Portland cement: a secondary level biocompatibility test. *J Indian Soc Pedod Prev Dent.* 2013;31(2):74-81. doi:10.4103/0970-4388.115698
33. Yaltirik M, Ozbas H, Bilgic B, Issever H. Reactions of connective tissue to mineral trioxide aggregate and amalgam. *J Endod.* 2004;30(2):95-99. doi:10.1097/00004770-200402000-00008
34. Bruno G de B, Alves APN, Menezes VA, Maia MCG, Bruno JA, Viana GS de B. Biocompatibility evaluation of an antibiotic paste after pulpotomy in dogs. *Braz J Oral Sci.* 2007;6(22):1397-1401. doi:10.20396/bjos.v6i22.8642999
35. Parirokh M, Mirsoltani B, Raoof M, Tabrizchi H, Haghdoost AA. Comparative study of subcutaneous tissue responses to a novel root-end filling material and white and grey mineral trioxide aggregate. *Int Endod J.* 2011;44(4):283-289. doi:10.1111/j.1365-2591.2010.01808.x
36. Nair PNR, Sjögren U, Krey G, Kahnberg K-E, Sundqvist G. Intraradicular bacteria and fungi in root-filled, asymptomatic human teeth with therapy-resistant periapical lesions: A long-term light and electron microscopic follow-up study. *J Endod.* 1990;16(12):580-588. doi:10.1016/S0099-2399(07)80201-9
37. Peters LB, van Winkelhoff A-J, Buijs JF, Wesselink PR. Effects of instrumentation, irrigation and dressing with calcium hydroxide on infection in pulpless teeth with periapical bone lesions. *Int Endod J.* 2002;35(1):13-21. doi:10.1046/j.0143-2885.2001.00447.x
38. Ghabraei S, Bolhari B, Sabbagh MM, Afshar MS. Comparison of Antimicrobial Effects of Triple Antibiotic Paste and Calcium Hydroxide Mixed with 2% Chlorhexidine as Intracanal Medicaments Against *Enterococcus faecalis* Biofilm. *J Dent Tehran Iran.* 2018;15(3):151-160.
39. Yadav RK, Tikku AP, Chandra A, Verma P, Bains R, Bhoot H. A comparative evaluation of the antimicrobial efficacy of calcium hydroxide, chlorhexidine gel, and a

curcumin-based formulation against *Enterococcus faecalis*. *Natl J Maxillofac Surg*. 2018;9(1):52. doi:10.4103/njms.NJMS_47_17

40. Sipert CR, Hussne RP, Nishiyama CK, Torres SA. In vitro antimicrobial activity of Fill Canal, Sealapex, Mineral Trioxide Aggregate, Portland cement and EndoRez. *Int Endod J*. 2005;38(8):539-543. doi:10.1111/j.1365-2591.2005.00984.x

41. Barcelos R, Santos MPA, Primo LG, Luiz RR, Maia LC. ZOE paste pulpectomies outcome in primary teeth: a systematic review. *J Clin Pediatr Dent*. 2011;35(3):241-248. doi:10.17796/jcpd.35.3.y777187463255n34

42. Holan G, Fuks AB. A comparison of pulpectomies using ZOE and KRI paste in primary molars: a retrospective study. *Pediatr Dent*. 1993;15(6):403-407.

43. Navit S, Jaiswal N, Khan SA, et al. Antimicrobial Efficacy of Contemporary Obturating Materials used in Primary Teeth- An In-vitro Study. *J Clin Diagn Res JCDR*. 2016;10(9):ZC09-ZC12. doi:10.7860/JCDR/2016/21883.8426

44. Coll JA, Sadrian R. Predicting pulpectomy success and its relationship to exfoliation and succedaneous dentition. *Pediatr Dent*. 1996;18(1):57-63.

45. Queiroz AM de, Assed S, Consolaro A, et al. Subcutaneous connective tissue response to primary root canal filling materials. *Braz Dent J*. 2011;22(3):203-211. doi:10.1590/s0103-64402011000300005

46. Chawla HS, Setia S, Gupta N, Gauba K, Goyal A. Evaluation of a mixture of zinc oxide, calcium hydroxide, and sodium fluoride as a new root canal filling material for primary teeth. *J Indian Soc Pedod Prev Dent*. 2008;26(2):53. doi:10.4103/0970-4388.41616

47. Sain S, J R, S A, George S, S Issac J, A John S. Lesion Sterilization and Tissue Repair-Current Concepts and Practices. *Int J Clin Pediatr Dent*. 2018;11(5):446-450. doi:10.5005/jp-journals-10005-1555

48. Alrahman MSA, Faraj BM, Dizaye KF. Assessment of Nitrofurantoin as an Experimental Intracanal Medicament in Endodontics. *BioMed Res Int*. 2020;2020:1-13. doi:10.1155/2020/2128473

49. Meropol SB, Chan KA, Chen Z, et al. Adverse events associated with prolonged antibiotic use. *Pharmacoepidemiol Drug Saf*. 2008;17(5):523-532. doi:10.1002/pds.1547

50. Rajsheker S, Mallineni SK, Nuvvula S. Obturating Materials Used for Pulpectomy in Primary Teeth- A Review. *J Dent Craniofacial Res*. 2018;03(01). doi:10.21767/2576-392X.100019

51. Kobayashi M, Tsutsui TW, Kobayashi T, et al. Sensitivity of human dental pulp cells to eighteen chemical agents used for endodontic treatments in dentistry. *Odontology*. 2013;101(1):43-51. doi:10.1007/s10266-011-0047-9.

53. James SL, Castle CD, Dingels ZV, Fox JT, Hamilton EB, Liu Z, Roberts NL, Sylte DO, Henry NJ, LeGrand KE, Abdelalim A. Global injury morbidity and mortality from 1990 to

2017: results from the Global Burden of Disease Study 2017. *Injury Prevention*. 2020 Oct 1;26(Supp 1):i96-114.

54. Chole RH, Gondivkar SM, Gadgil AR, Balsaraf S, Chaudhary S, Dhore SV, Ghonmode S, Balwani S, Mankar M, Tiwari M, Parikh RV. Review of drug treatment of oral submucous fibrosis. *Oral oncology*. 2012 May 1;48(5):393-8.

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