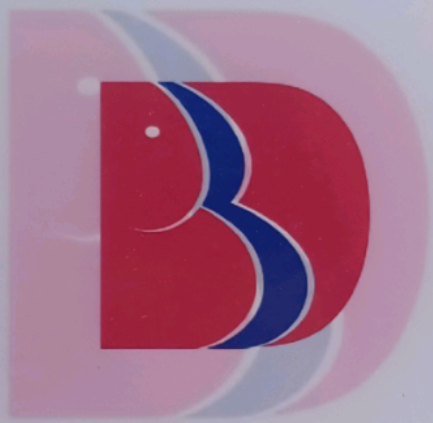
"Comparative evaluation of antimicrobial efficacy of Chemical, Bio-material and Herbal irrigation solution in primary teeth: An *in vivo* study"

BABU BANARASI DAS UNIVERSITY, LUCKNOW

Thesis submitted in partial fulfillment of the requirements for degree of

MASTER OF DENTAL SURGERY



In the subject of

PEDIATRIC AND PREVENTIVE DENTISTRY

DEPARTMENT OF PEDIATRIC AND PREVENTIVE DENTISTRY

BABU BANARASI DAS OF DENTAL SCIENCES, LUCKNOW, UTTAR PRADESH- 226028

BATCH- 2021-2024

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Comparative evaluation of antimicrobial efficacy of Chemical, Bio-material and Herbal irrigation solution in primary teeth: An in vivo study "** is a bonafide and genuine research work carried out by me under the guidance of **DR.SOMYA GOVIL, Reader,** Department of Paediatric and Preventive Dentistry, Babu Banarasi Das College of Dental Sciences, Babu Banarasi Das University, Lucknow, Uttar Pradesh.

Date: 10-02-24

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CERTIFICATE

This is to certify that the dissertation entitled **"Comparative evaluation of antimicrobial efficacy of Chemical, Bio-material and Herbal irrigation solution in primary teeth: An *in vivo* study"** is an original bonafide research work done by **Dr.AJAY KUMAR YADAV,** in partial fulfillment of the requirement for the degree of **MASTER OF DENTAL SURGERY (M.D.S)** in the speciality of **PEDIATRIC AND PREVENTIVE DENTISTRY** under our supervision.

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Dr. AJAY KUMAR YADAV, himself in this department. The candidate fulfills all the conditions necessary for the submission of this thesis.

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ANNEXURE 7

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Author Name	AJAY KUMAR YADAV
Title	Comparative evaluation of antimicrobial efficacy of Chemical, Bio-material and Herbal irrigation solution in primary teeth: An in vivo study
Paper Submission ID	1425134
Submitted by	amarpal.singh056@bbdu.ac.in
Submission Date	2024-02-12 15:57:03
Total Pages	10
Document type	Dissertation

Result Information

Similarity **0 %**

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Database Selection

Language	English
Student Papers	Yes
Journals & publishers	Yes
Internet or Web	Yes
Institution Repository	Yes

***

ACKNOWLEDGEMENT

“Recognizing the truth is the foundation of all wisdom." My three years after graduation have been a journey of learning for me. "Thank you" are insufficient to express my deep appreciation and respect for the people who made this research possible. I am grateful for their diverse contributions and support during the creation of my dissertation and I am honored to take this chance to acknowledge them.

“I extend my sincere thanks to my advisor and mentor, **Dr. Somya Govil**, for her constant support and guidance. Her insightful advice helped me navigate the challenges of this research project. I am deeply grateful for their patience, motivation, and expertise that she shared with me. Most importantly, I am thankful for support, collaboration, and mentorship throughout the past three years."

Above all, I would like to thank the head of the family **Dr. Monika Rathore**, Professor and Head of the Department for her unflinching support, advice and brilliant supervision. Her valuable suggestions and her instructions have served as the major contributor towards the completion of this project.

I am extremely thankful to **Dr. Neerja Singh** and **Dr. Subash Singh for** their kind cooperation and helping me with the conductance of my dissertation, without their constant support this dissertation would not have been possible.

I am grateful for the love and support of my parents **Shre.Jilajeet yadav** and mother **Shrimati. Urmila Yadav** who have been my sources of strength. I would also like to expressmy gratitude to my brother **Cadet.Ashok yadav and Dr.Arvind yadav** who have been a constant source of support and encouragement.

I would also like to enormously thank to **Dr. Mansi Semwal**, and **Dr. Bhibhav Dube**y for their valuable time, knowledge and guidance throughout these three years. Their incessant encouragement and professional insight have helped me to learn in the right direction by leaps and bounds.

Finally, I will be failing in my duty if I do not thank whose profound help, support and sincerity has helped me in completing my thesis. I deeply appreciate the undying support from my dear collegues **Dr. Sarwani Mishra ,Dr. Aayushi Bharadwaj, Dr. Ninapyari Ahanthem**, and **Dr. Sadia Salman.**My journey in this

department would be incomplete without their encouragement and patience through the constant highs and lows.

I also extend my heartfelt gratefulness to my seniros **Dr. Needhi Singh ,Dr. Raunaq Sahu, Dr. Akash Roy Chowdhury, Dr. Saheli Basu** and my junior s **Dr. Shreeja Anand ,Dr. Anushka Banerjee, Dr. Spandan Dey, Dr. Sushmita Gupta,** and **Dr. Shifa Amir**

A special thanks to my friends **Dr. Abhishek mishra (Microbiologist),** and **Dr. Abhijeet humne (Oral surgery)** who have always been my side and supported me throughout my journey.

It is a pleasure at this point to thank my principal, **Dr. Puneet Ahuja** for providing me with the opportunity to conduct the research and use the facilities of this college.

I would like to express my heartfelt gratitude to the non-teaching personnel in the Pediatric Department for their compassion and assistance.

This is dedicated to my loved ones, friends, and instructors who have supported me throughout my journey. Without them, this would have remained just a dream.

Dr. Ajay Kumar Yadav

ABSTRACT

Background:

Successful root canal therapy relies on the combination of proper instrumentation, irrigation and obturating of the root canal. The root canal morphology of primary teeth has bizarre internal geometry and features like internal connections and horizontal anastomoses, which makes debridement of necrosed pulp difficult only with mechanical instruments. Therefore, chemical irrigants play a major role to reach to these accessory canals and their ramifications and flush out debris, dissolves tissue and disinfect them. The potential side effects and safety concerns of conventional Endodontic irrigants have led to upheaval in use of various alternatives.

Aim:

The aim of study is to evaluate the antimicrobial efficacy of three different irrigating solutions in primary teeth.

Methodology:

Children aged 4-9 years were selected for the study. A total of 75 Child patients were included in this study according to inclusion criteria. After the endodontic access opening of a primary molar , fluid in the chamber was collected using a sterile paper point as pre-sample (S1) from the pulp chamber and the largest diameter canal (Distal/Palatal). The sterile paper point was kept in contact for 1 minute, and then cautiously obtained sample S1 was placed in a sterile collection tube filled with sterile saline in a 1.5-ml tube that had been disinfected prior with iso-propyl alcohol. The Endodontic irrigation sequence was performed by irrigating with normal saline, followed by pulp debridement. Final irrigation was performed with the study irrigant for 5 minutes and another sample (S2) was collected from the largest canal as the same procedure, sampling was done for pre-sample (S1). Samples were transferred to a storage box and transferred immediately to the microbiological lab for analysis.

Result:

There was a significant reduction of microbial CFU count in inter-group and intra-group comparison of NaOCl , Chitosan NP, and liquorice groups. Overall NaOCl showed the highest reduction in microbial CFU count followed by Chitosan And the least reduction was seen in Liquorice.

INTRODUCTION

Primary teeth are essential for harmonious occlusion growth, arch length preservation, and functional activity like chewing and speech in children [1]. Endodontic infections are caused by opportunistic microorganisms, which invade the deciduous tooth root canals, causing necrotic tissue and beginning an infectious process. Eliminating these microorganisms from the root canal should be the objective of treatment. A pulpectomy is a non-vital pulp therapy for primary teeth. It involves removing infected pulp tissue, debris, and microorganisms with an irrigant and obturating the canals with an appropriate material.

A diverse range of poly-microbial species, including aerobic, anaerobic, and facultative microorganisms, characterizes endodontic infections [2]. Significant differences were found between the root canal microorganisms recovered from asymptomatic teeth and also which is isolated from clinically symptomatic teeth. Prevailing bacterial species found in primary teeth with infected root canals include E. faecalis, Porphyromonas gingivalis, and Treponemadenticola, with E. faecalis present in 63% of necrotic primary teeth [3]. In primary root canal infections, the highest concentration of microorganisms has been detected in the deeper regions of lateral canals, apical implications, and dentinal tubules.

In Pediatric Endodontics, irrigants are essential due to the unique anatomy of primary teeth, which often have curved and convoluted root canals [4]. While current treatments have made significant progress in addressing intra-canal infections, there remains a need for further advancements to eliminate all microorganisms and prevent the possibility of root canal re-infection. When choosing an irrigant for pulpal therapy in primary teeth, it is crucial to consider the differences among the dentin substrate and avoid irritating the periapical tissues. Additionally, care should be taken to avoid harming the germ of the permanent successor tooth, as physiologic root resorption can lead to the apical extrusion of the endodontic irritants [5,6].Chemical irrigating solutions can be cytotoxic and, if extruded into the periapical tissues, may cause mild pain [7]. It is intriguing to discover that normal saline is a standard irrigating solution in numerous endodontics and surgical procedures. Given its isotonic nature to bodily

fluids, it is deemed safe and has no adverse effects, even if inadvertently pushed into the periapical tissues. Nevertheless, it is imperative to remember that saline cannot be the sole solution utilized as an irritant. It should be incorporated in conjunction with or alternative with other solutions, such as sodium hypochlorite, to guarantee efficacious treatment.

Sodium hypochlorite (NaOCl) is a chemical irrigant for root canal disinfection. Numerous studies have revealed its strong antibacterial properties and ability to break down pulp. Sodium hypochlorite is used in concentrations varying from 0.5% to 5.25%; it is a potent antimicrobial agent and effectively dissolves pulpal remnants and organic components of dentine. It is used as an unbuffered solution at pH 11 in 0.5–5.25% concentrations and buffered with bicarbonate buffer (pH 9.0), usually as a 0.5% solution (Dakin's solution) [8]. Despite these factors, a 3% w/v concentration of sodium hypochlorite remains popular due to its minimal tissue toxicity [9]. Additionally, it may not consistently disinfect the root canal and potentially harm the permanent tooth follicle, peripheral tissues, and oral mucosa in pediatric patients.

Chitosan-based nanoparticles, derived from chitin, a natural bio-material, have shown significant potential for various biomedical applications. Due to their broad-spectrum antimicrobial activity, chitosan nanoparticles are a promising candidate for endodontics [10]. These cationic nanoparticles react with bacterial cells' negatively charged surface, leading to cell death. Additionally, they are more tissue-friendly than other solutions [11]. Ongoing research will result in novel bio-materials with enhanced functionality and efficacy. However, there needs to be more research on the in-vivo antimicrobial activity of chitosan nanoparticles; their potential as an endodontic irrigation solution in primary root canals for their antimicrobial activity warrants further investigation.

Herbal products are known for their high antimicrobial activity, bio-compatibility, anti-inflammatory, and anti-oxidant properties. Such properties have led to a significant increase in the use of herbal products in endodontic treatment. Glycyrrhiza glabra, commonly known as Liquorice or mulethi, is approved as a safe food flavoring and sweetening agent [12]. Recent research has shown that Liquorice has potential therapeutic benefits for treating oral diseases such as dental caries due to its anti-adherence, antimicrobial, and anti-inflammatory properties. Liquorice is commonly used in kampo medicine and has several pharmaceutical effects, including anti-inflammatory, antiviral, and anticarcinogenic properties. Liquorice and its bioactive constituents may hold promise as alternative therapeutic agents for oral disease treatment [13].

Aim & Objectives of The Study

AIM

Aim of study is to evaluate the antimicrobial efficacy of three different irrigating solution in primary teeth.

OBJECTIVES

To evaluate the antimicrobial efficacy of sodium hypochlorite, liquorice and chitosan as root canal irrigants in primary teeth.

To compare the antimicrobial activity of sodium hypochlorite ,liquorice and chitosan as root canal irrigants in primary teeth.

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REVIEW OF LITERATURE

A literature review is a written summary of important works and other materials on a particular subject. Scholarly journal articles, books, government reports, Web sites, and other sources may be used in the review. The literature review provides a description, summary, and evaluation of each source.

IRRIGANTS

SODIUM HYPOCHLORITE (NAOCL)

NaOCl is the most commonly used irrigating solution used in dentistry[14]. NaOCl gives rise to sodium and hypochlorite ions when combined with water, thereby establishing equilibrium with hypochlorous acid, which is responsible for the antibacterial activity. It can also dissolve organic components such as pulpal remnants and collagen.

D'Arcangelo C., Varvara G., and De Fazio P. (1999)[15] compared and evaluated the action of different root canal irrigants on Enterococcus fecalis. The irrigants used 3% sodium hypochlorite, 0.2% chlorhexidine, and 0.2% cetrimide. In three test tubes, each irrigant was kept in contact with the bacterial species used for the experiment and suspended in Brain Heart Infusion broth for 10, 20, or 30 min. Results showed that all irrigants had a bactericidal effect on Enterococcus fecalis. The most significant bactericidal effect was demonstrated by 3% sodium hypochlorite, followed by 0.2% chlorhexidine, and then the least bactericidal effect by 0.2% cetrimide.

Retamozo B, Torabinejad M, and Shabahang S (2010)[16] conducted a study to determine the concentration of sodium hypochlorite and the irrigation time required to disinfect dentin cylinders infected with Enterococcus fecalis. The results showed that the most effective irrigation regimen was 5.25% at 40 minutes. In contrast, irrigation with 1.3% and 2.5% NaOCl for this time interval was ineffective in removing E. fecalis from infected dentin cylinders.

Poonam Shingare. (2011)[17] conducted a study comparing the antimicrobial activity of

sodium hypochlorite with the antibacterial activities of propolis and miswak. This study included 40 infected primary teeth (20 male and 20 female patients). The subjects were divided into four groups of 10 children. Group 1 received 3% sodium hypochlorite as an irrigating solution; Group 2 received 12.5% alcoholic extract of miswak; Group 3 received 11% alcoholic extract of propolis; and Group 4 received 0.9% saline. Pre- and post-irrigation samples using sterile paper points were cultured on tryptose soya agar at 37 oC for 24-48 hours. The colonies were counted with a digital colony counter. The differences in pre- and post-irrigation values were calculated for each group, with the most significant difference being seen in group 1 (95.549%), followed by group 2 (89.794%), group 3 (34.735%), and group 4 (28.087%). It was concluded that sodium hypochlorite irrigating solution has the highest antibacterial activity among other herbal irrigating solutions.

Balakrishnan A., Sam JE., and Kumar A. (2012)[18] evaluated in their Study the Antibacterial efficacy of Triphala, Morinda citrifolia, Aloe Vera, vitex negundo, and sodium hypochlorite against Enterococcus fecalis. The agar disk diffusion method was used to study the antibacterial efficacy of the irrigants. Sterile discs containing the Herbal irrigants were placed on the agar plate containing the bacterial culture. The results showed that 5.25% sodium hypochlorite was the most efficient, followed by Triphala, morinda citrifolia, aloe vera, and Vitex negundo in decreasing antibacterial efficacy.

Tulsani SG., Chikkanasarah N., and Bethur S. (2014)[19] compared the in vivo antimicrobial efficacy of NaOCl 2.5% and Bio-pure MTADTM using the conventional needle irrigation method against Enterococcus fecalis in 40 nonvital single-rooted primary teeth of children aged 4–8 years. The sample was divided into three groups according to the irrigants, with 0.9% saline as the control group. Paper point samples were taken before irrigation (S1) and after irrigation (S2) and were evaluated using real-time PCR. There was a significant difference in the antimicrobial activity efficacy of NaOCl 2.5% and Bio-pure MTADTM compared to 0.9% saline.

Chandrappa PM., Dupper A., Tripathi P., Arroju R., Sharma P., Sulochana K. (2015)[20] Determined the antimicrobial activity of herbal medicines and chlorhexidine against Enterococcus faecalis in sixty agar plates. The plate samples were divided into groups with 15 samples: Group I: chlorhexidine 2%; Group II: neem extract; Group III: tulsi extract; and Group IV: distilled water. The microbiological procedure was carried out, and the results concluded that herbal medicines were effective against E. faecalis compared to 2% chlorhexidine gluconate.

Chhabra N., Gyanani H., and Kamatagi L. (2015)[21] evaluated the effectiveness of the combination of two natural extracts in varying ratios for the removal of the smear layer either alone or supplemented with sonic agitation (EndoActivator) in 50 extracted single-rooted permanent teeth. The teeth were divided into six groups according to various irrigants. They concluded that the group with EDTA irrigant showed the best results, whereas the combination of two extracts in a 2:1 ratio was slightly better than the 1:1 ratio. The smear layer removal was more efficient when accompanied by sonic agitation.

Podar R., Kulkarni GP., Dadu SS., Singh S., and Singh SH. (2015[22]) evaluated and compared the antimicrobial efficacy of 6% Morindacitrifolia (noni berry), Azadirachta indica (neem), and 3% sodium hypochlorite (NaOCl) as root canal irrigants in thirty nonvital maxillary anterior. Pre-operative paper point samples were taken, followed by post-operative samples after three days. The samples were sent to the lab for a CFU count. They concluded there was no difference in the antimicrobial efficacy of 6% M. citrifolia, 3% A. indica, and 3% NaOCl as root canal irrigants.

Joy Sinha D., D S Nandha K., Jaiswal N., Vasudeva A., Prabha Tyagi S., and Pratap Singh U. (2015)[23] assessed the antibacterial effect of Azadirachta indica (Neem) or Curcuma longa (Turmeric) against Enterococcus faecalis Compared with that of 5% sodium hypochlorite or 2% chlorhexidine in vitro. The activity of neem, chlorhexidine, sodium hypochlorite, or turmeric against E. faecalis was measured on agar plates using the agar diffusion method. Chlorhexidine or sodium hypochlorite against E. faecalis suggests a promising alternative to

the other root canal irrigants tested..

Saxena D., Saha SG., Saha MK., Dubey S., Khatri M. (2015)[24] evaluated the antimicrobial activity of five herbal extracts and compared their activity with 2.5% sodium hypochlorite against Enterococcus faecalis. E. faecalis was inoculated onto a brain-heart infusion agar plate. Discs impregnated with herbal medicaments were further assessed with lab procedures. They concluded that propolis and AI have significant antimicrobial activity against E. faecalis.

Dhariwal NS. (2016)[25] selected 30 patients, and samples were taken from the root canals of infected primary teeth using sterile absorbent paper points and transferred to tubes containing a thioglycolate transport medium. The bacteria were then isolated using standard microbiological protocols and subjected to antibiotic sensitivity testing using the three test irrigants. They concluded that the most commonly isolated bacteria included Porphyromonas species, Bacteroides fragilis, Pepto streptococcus, and Staphylococcus aureus. Sodium hypochlorite and Curcuma longa (turmeric) showed sound antibacterial effects and were effective against most isolated bacteria.

Hegde RJ. and Bapna K. (2016)[26] compared the removal of the endodontic smear layer using ethylene glycol bis (beta-amino ethyl ether)-N, N, N'-tetra acetic acid and citric acid in 30 extracted primary teeth. The teeth were divided into three groups; the third was the control group (saline). They were subjected to a scanning electron microscope after being split longitudinally. The results advocate that sequential irrigation of canals with 17% EGTA followed by 5% NaOCl produces efficacious and smear-free root canal walls.

Chandwani M., Mittal R., Chandak S., and Pimpale J. (2017)[27] proposed a randomized comparative in vivo study to evaluate the microbial reduction in 60 deciduous molars among children aged 6–9 years using Morinda citrifolia juice and NaOCl. The sample was divided into two groups based on irrigating solutions. Paper point samples were collected pre-irrigation (S1) and post-irrigation (S2) and were transferred for microbial assay. In the intra-group comparison, both groups showed a statistically significant reduction, whereas it did not show a statistically significant reduction when an intergroup comparison was carried out between the

two groups.

RM., Patil PH., Gulve MN., Kolhe SJ., Samuel RM., Aher GB. (2018)[28]An in-vitro study compared the smear layer removal efficacy of etidronic acid-based irrigating solution with others in the apical third of the single-rooted mandibular premolar teeth. After biomechanical preparation, the samples were randomly divided into four groups (n = 10): Group I: normal saline (negative control); Group II: 5.25% sodium hypochlorite (NaOCl) with surfactant and 17% ethylenediaminetetraacetic acid (EDTA) with surfactant; Group III: freshly mixed BioPure MTAD; and Group IV: freshly mixed Chloroquick solution. After the final irrigation, the teeth were split into two halves and observed under a scanning electron microscope. 5.25% NaOCl with surfactant and 17% EDTA with surfactant were found to be more efficient than MTAD and chloroquick in the removal of the smear layer in the apical third of the root canal.

Batinić M., Ročan M., Budimir A., Anić I., Bago I. (2018)[29] compared the final disinfection protocols using antimicrobial photodynamic therapy and different irrigants in 68 extracted mandibular human single canal teeth. The teeth were inoculated with Enterococcus faecalis and divided into four groups and irrigated as follows: Group 1. 2.5% NaOCl and EDTA, followed by the application of the aPDT; Group 2. 2.5% NaOCl, EDTA, and 2.5% NaOCl; Group 3. 2.5% NaOCl and QMIX solution; and Group 4. 2.5% NaOCl and EDTA in the control group. They concluded that a PDT used after irrigation with NaOCl and EDTA demonstrated similar antimicrobial efficacy as conventional irrigation with NaOCl.

Demirel A., Yüksel BN., Ziya M., Gümüş H., Doğan S., and Sari Ş. (2019)[30] proposed a study to compare the efficacy of different irrigation protocols on smear layer removal in root canals of 40 extracted maxillary primary incisor teeth by scanning electron microscopy (SEM). The samples were divided into four groups: 1% sodium hypochlorite (NaOCl), 10% ethylenediaminetetraacetic acid (EDTA) + 1% NaOCl, 6% citric acid (CA) + 1% NaOCl, and 0.9% physiological saline (PS). After the irrigation procedures, root canal walls were examined by SEM. They concluded that 10% EDTA+1% NaOCl and 6% CA+1% NaOCl could be alternative irrigation protocols regarding smear layer removal. However, due to the absence of erosive dentinal changes, it might be suggested that 6% CA+1% NaOCl can be recommended compared to 10% EDTA+1% NaOCl in primary root canals.

Walia V., Goswami M., Mishra S., Walia N., and Sahay D. (2019)[31] evaluated the difference in the antibacterial efficacy of different irrigants in sixty primary tooth root canals. The samples were divided into groups: group 1 (2% chlorhexidine), group 2 (1% sodium hypochlorite), group 3 (laser irradiation), and group 4 (saline). Pulp tissue was extirpated from the canals, and the pre-and post-operative paper point samples were obtained again and sent for microbiological examination. They concluded that 2% chlorhexidine, 1% sodium hypochlorite, and laser irradiation reduced the root canal infection. Hence, diode laser irradiation may supplement existing protocols for disinfecting the root canal system.

Mali S., Singla S., Tyagi P., Sharma A., Talreja N., and Gautam A. (2020)[32] compared the efficacy of different herbal irrigants on removing the smear layer of forty extracted single-rooted primary teeth. The sample teeth were allocated randomly into four groups of ten each: Group 1 (NaOCl), Group 2 (Nutmeg), Group 3 (Myrobolan), and Group 4 (Tulsi). Teeth samples were decorated at the level of the cementoenamel junction, followed by appropriate irrigation. The samples were examined using a scanning electron microscope. They concluded that tulsi, nutmeg, and myrobolan can be effectively used as an irrigant in primary teeth, with tulsi demonstrating the better of the three irrigants.

Ruksakiet K. (2020)[33], in a systematic review, compared the antimicrobial efficacy of chlorhexidine (CHX) and sodium hypochlorite (NaOCl) and found that both CHX and NaOCl can reduce bacterial infections after irrigation without any significant difference in antimicrobial efficacy between them.

Agnihotri A. (2020)[34] reviewed herbs such as Tulsi, Miswak, and M. Citrifolia. He concluded that all herbs deficiency could potentially replace the chemical irrigants in pediatric dental patients. This herbal irrigant could be used as a routine root canal irrigant and as an adjunct to chemical irrigants, lowering their dose and toxicity in resistant or failed root canal treatment cases.

CHITOSAN NANOPARTICLE

Chitosan nanoparticles exhibit significant antibacterial activity in in-vivo settings due to their higher surface area and charge density, which enable them to react with the negatively charged surface of bacterial cells, ultimately resulting in bacterial cell death[35].

Kishen (2008)[36] investigated the antibacterial and antibiofilm efficacy of cationic nanoparticles for root canal disinfection. Experiments were performed in two stages. In stage 1, experiments were conducted to examine the physical properties of three types of nanoparticles. The antibacterial properties of nanoparticles alone and nanoparticulates mixed with zinc oxide-eugenol-based sealer were studied. In stage 2, the ability of nanoparticulate-treated dentin to prevent bacterial adherence was examined. Zinc oxide nanoparticles, chitosan nanoparticles, a mixture of zinc oxide and chitosan nanoparticles, and zinc oxide nanoparticles with a multilayered coating of chitosan were tested. This study showed that the incorporation of nanoparticles did not alter the flow characteristics of the sealer but improved the direct antibacterial property and the ability to reach out to antibacterial components. There was a significant reduction in the adherence of Enterococcus faecalis to nanoparticulate-treated dentin ($p < 0.05$). All the nanoparticles tested showed bacterial killing, and the killing rate depended on the time and concentration used. CS-NP showed the complete killing of bacteria after 8 hours.

P V Silva., D F C Guedes., F V Nakadi., J D Pécora., and A M Cruz-Filho. (2012)[37] conducted a study to evaluate the efficacy of smear layer removal using Chitosan compared with different chelating agents and to quantify, by atomic absorption spectrophotometry with flame (AASF), the concentration of calcium ions in these solutions after irrigation. The teeth were randomly divided into groups (n = 5) according to the type of final irrigation: 15% EDTA, 0.2% chitosan, 10% citric acid, 1% acetic acid, and control (without last irrigation). The total volume of each chelating solution was collected from the canals and analyzed by AASF for the quantification of calcium ions in the solutions. Chitosan: The study concluded that 15% EDTA, 0.2% chitosan, and 10% citric acid effectively removed the smear layer from the middle and apical thirds of the root canal. 15% EDTA and 0.2% chitosan were associated

with the most significant effect on root dentine demineralization, followed by 10% citric acid and 1% acetic acid.

Shrestha A, Kishen A, et al (2014)[38] evaluated the antibacterial effect of chitosan nanoparticles with a photosensitizer against Enterococcus fecalis. The following Agents were tested for theiririr inhibitory effects. 28 mg dentin powder; 10 mg fresh
bovine pulp, frozen and powdered; 5 mg dentin-matrix; and 2% and 18% BSA and LPS (1 mg/mL). Extracted human third molars and bovine teeth from the slaughterhouse were obtained. The experiments were carried out in colony-forming unit counts with three samples per group each time. The results proved that the inherent antibacterial activity of polycationic chitosan nanoparticles and the singlet oxygen released after photoactivation of the photosensitizer synergistically provided chitosan particles the potential to achieve significant antibacterial efficacy even in tissue inhibitors within root canals.

Del Carpio-Perochena A., Bramantel C.M., and Duarte M.A.H. (2015)[39]investigated the ability of bioactive chitosan nanoparticles (CNPs) to remove the smear layer and inhibit bacterial recolonization on dentin. According to the treatment, one hundred bovine dentin sections were divided into five groups (n = 20 per group). The irrigating solutions were 2.5% sodium hypochlorite (NaOCl) for 20 min, 17% ethylenediaminetetraacetic acid (EDTA) for 3 min, and 1.29 mg/mL Chitosan Nanoparticles for 3 min. The samples were irrigated with either distilled water (control), NaOCl, NaOCl-EDTA, NaOCl-EDTA-CNPs, or NaOCl-CNPs. After the treatment, half of the samples (n = 50) were used to assess the chelating effect of the solutions using portable scanning electronic microscopy. In contrast, the other half (n = 50) were infected intra-orally to examine the post-treatment bacterial biofilm-forming capacity. It was concluded that CNPs could be used as a final irrigant during root canal treatment, with the dual benefit of removing the smear layer and inhibiting bacterial recolonization on root dentin.

Babu B., Nair R.S., and Angelo J.M.C. (2017)[40] evaluated the efficacy of chitosan silver nanocomposite on candida albicans when compared to three different antifungal agents—fluconazole, clotrimazole, and amphotericin B—in combination with the standard irrigation

protocol of 5.25% NaOCl and 17% EDTA. Fifty experimental teeth were biomechanically prepared and inoculated with a suspension of C. albicans. At 96 hours, teeth were divided into five experimental groups. The groups were treated with respective irrigating solutions for 1 minute. An inoculation loop was used to remove aliquots from the fluid, plated on 4% Sabouraud Dextrose Agar, and incubated for 48 hours. After incubation, the growth of C. albicans was assessed with light microscopy at ×400. The data were statistically analyzed. The colony-forming unit (CFU) was determined for all five groups. It was concluded that chitosan-silver nanocomposite as an endodontic irrigant can inhibit the growth of C. albicans in combination with a standard irrigation protocol.

Mohamed I. Elshinawy., Lamiaa A. Al-Madboly., Walaa M. Ghoneim., and Nehal M. El-Deeb. (2018)[41] conducted a study to investigate the antimicrobial-biofilm activity of chitosan (Ch-NPs), silver nanoparticles (Ag-NPs), and ozonated olive oil (O3-oil) either separately or combined against endodontic pathogens. While testing the antimicrobial activity, Ch-NPs showed the most minor minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values, exerting eightfold higher bactericidal activity than O3-oil against both Enterococcus faecalis and Streptococcus mutans, as well as fourfold higher fungicidal activity against Candida albicans. The safety pattern results showed that O3-oil was the safest compound, followed by Ch-NPs. The double combination of Ch-NPs and O3-oil reduced the mature, viable biofilm on premolars in an ex vivo model by 6-log reductions, with a fast kill rate, indicating potential use in treating root canals. Therefore, the double combination can potentially eradicate mature mixed-species biofilms and, hence, is potent, novel, and safe.

Polliana Vilaça Silva Antunes., Luis Eduardo Souza Flamini ., and Jardel Francisco Mazzi Chaves., Ricardo Gariba Silva. (2019)[42] compared the effects of final canal irrigation with Chitosan and EDTA on dentin microhardness, sealer dentin tubule penetration capacity, and push-out strength. Fifty canine roots were distributed according to the final irrigation protocol (n = 10): G1: 15% EDTA with conventional irrigation; G2: 15% EDTA with Endovac; G3: 0.2% chitosan with traditional irrigation; G4: 0.2% chitosan with Endovac; and G5: without irrigation. They concluded that 0.2% chitosan and 15% EDTA solutions act

similarly about the variables studied

Kondreddi N., Venigalla BS., and Singh TV. (2020)[43] compared the antibacterial activity of 0.2% chitosan, 5.25% NaOCl, 2% chlorhexidine, and alternating solutions of chitosan, sodium hypochlorite, and chlorhexidine against Enterococcus fecalis. Sixty extracted single-rooted teeth were selected and used for the study. After incubation with Enterococcus fecalis, samples were divided into six groups according to the solutions used for irrigation, that is, CHX, NaOCl, Chitosan, an alternating solution of Chitosan and hypochlorite, an alternating solution of Chitosan and CHX, and saline. Antibacterial efficacy was assessed by obtaining the samples from the root canal before and after the irrigation using paper points, culturing them on blood agar plates, and measuring the number of colony-forming units (CFUs) formed. The results revealed maximum antibacterial activity when using chitosan instead of CHX and NaOCl. Independently, hypochlorite showed total antibacterial activity, followed by CHX and chitosan, which showed almost similar antibacterial activity.

Sonam Dahil., Rakesh Mittal., and Monika Tandon (2021)[44] evaluated and compared the antimicrobial efficacy of two herbal products as root canal irrigants in primary endodontic infections. Seventy-eight patients were selected, out of which only 66 met the inclusion criteria and were further randomly divided into three groups (n = 22 each): Group-1: 2.5% Sodium Hypochlorite, Group 2: Chitosan, and Group 3: PropolisThree samples were taken for each tooth. Biomechanical preparation was done up to the master apical size #40 K-file. Irrigation was done with the respective irrigants. The post-instrumentation sample (S2) was similar to S1. Microbiological samples (S1, S2) were preincubated for 30 minutes and plated on brain heart infusion agar. Colonies were counted after 24 hours using the classic bacterial counting method. The study concluded that herbal products have shown significant antimicrobial activity compared to 2.5% sodium hypochlorite in primary endodontic infections in patients and can be recommended for use in clinical situations.

Abiding T, Susilo D, and Gani BA. (2022)[45] conducted a study to evaluate the efficiency of 0.2% nanochitosan as a root canal irrigant against E. fecal. The experimental design was a

randomized block design with a total number of 27 teeth divided equally into three treatment groups, namely, Group A: 0.2% high molecular nano-chitosan + PUI after remodeling treatment of root canals; Group B: 2.5% NaOCl with additional PUI after remodeling root canal treatment; and Group C: distilled water solution + PUI after remodeling root canal treatment. Following the treatments, the growth of E. fecalis and the surface roughness of the tooth root canal were assessed. They concluded that the irrigation of root canals with 0.2% high-molecular-weight nanochitosan had significant antibacterial activities against E. fecalis.

Goel P, Galhotra V, and Makkar S. (2022)[46] conducted an in vitro study to compare the antibacterial efficacy of 0.2% chitosan, 3% sodium hypochlorite, and 2% chlorhexidine against Enterococcus fecalis. The root canals of 72 extracted intact human single-rooted teeth with single canals were prepared, and E. fecalis was incubated in the root canals for seven days. The teeth were then randomly divided into the following four experimental groups: group I: saline, group II: 0.2% chitosan, group III: 3% sodium hypochlorite, and group IV: 2% chlorhexidine. The effect of each irrigant was evaluated by counting the number of colony-forming units observed on inoculation. The results of the study showed that the maximum number of colony-forming units was observed in group I: saline (106.83 CFU/mL), followed by group III: 3% sodium hypochlorite (54.33), group II: 0.2% chitosan (0.50 CFU/mL), and group IV: 2% chlorhexidine (0 CFU/mL).

LIQUORICE

Liquorice (Glycyrrhiza glabra), known as Atimaturam, is the most widely used flavoring agent and crude drug in kampo medicines (traditional Chinese medicines modified in Japan). The compound glycyrrhizin (or glycyrrhizic acid), found in liquorice, is antiviral, antimicrobial, anti-inflammatory, and anti-carcinogenic[47].

Vivek K. Gupta. (1998)[48] evaluated the antimicrobial activity of the roots of Glycyrrhiza glabra. The results showed that it had antimycobacterial activity and also inhibited growth. Bodet C. (2005) assessed the effect of Glycyrrhiza uralensis (Licorice) on the reduction of periodontopathogen-induced inflammatory responses. Licorice-treated Monocyte-derived macrophages were induced by the lipopolysaccharide (LPS) of Aggregatibacter

actinomycetemcomitans and Porphyromonas gingivalis. Enzyme-linked immunosorbent assays were used to detect the levels of tumor necrosis factor-alpha (TNFalpha) and interleukin (IL)-1 beta, -6, and -8. The licorice extract showed good anti-inflammatory properties. The licorice extract inhibited the phosphorylation of macrophage intracellular signaling. The results indicated that licorice extracts of Gram-negative and Gram-positive bacteria could arrest periodontitis-associated tissue destruction. The results showed that licorice could be used as an antitubercular agent.

Segal R. (2006)[49] evaluated liquorice and its component glycyrrhizin's ability to inhibit the growth of cariogenic Streptococcus mutans and its adherence to glass. Neither of the test materials promoted growth. In the presence of sucrose, glycyrrhizin inhibited plaque formation. The concentration at which glycyrrhizin showed complete inhibition was 0.5–1%. The results concluded that glycyrrhizin could be used effectively for topical oral medications.

Haraguchi . (2006)[50] evaluated the antimicrobial activity of retrochalcones, that is, echinatin and Licochalcone A-D, which were isolated from Glycyrrhiza inflata roots.
Gram-positive bacteria were inhibited at a higher rate by licochalcone A, and C. Retrochalcones inhibit oxygen consumption in bacterial cells. Licochalcones inhibited the NADH-cytochrome c reductase but did not deter the cytochrome c oxidase. In bacteria's respiratory electron transport chain, lignocarbacones inhibited the site between CoQ and cytochrome.

Söderling E. (2008)[51] evaluated the effect of starch gel containing lignin on the accumulation of plaque and its microbial composition. The plaque was evaluated for facultative bacteria, total streptococci, and Mutans streptococci by plate culturing. PCR denaturing gradient gel electrophoresis was used to determine the bacteria's stability in the plaque. The results indicated that neither control gel (8% acid-hydrolyzed corn starch) nor licorice gel differed in the microbial counts. The stability of bacterial populations was not affected by licorice gel. To conclude, licorice extract with starch gel did not affect plaque formation after consuming licorice gel for two weeks.

A. E. Badr (2011)[52] studied Licorice's cytotoxic and antimicrobial activity and compared it with the calcium hydroxide Ca (OH) 2 by conducting broth microdilution tests, agar-well diffusion methods, and biofilm susceptibility assays. The results indicated that liquorice did not have lethal effects on human periodontal ligament fibroblast cells, and it had potent antimicrobial activity against Enterococcus faecalis.

Eesha Jain. (2013)[53] conducted the study to evaluate the cariostatic efficacy of ethanolic and aqueous liquorice extracts in vitro and in vivo. A double-blind pilot study was conducted among pediatric patients aged 7–14. They are divided into three groups. Group 1: chlorhexidine gluconate (0.156%) mouthwash; Group 2: aqueous licorice mouthwash (15%); and Group 3: ethanolic licorice mouthwash (3.75%). The saliva samples were evaluated for streptococci mutans colony counts and pH change. The results concluded that ethanolic and aqueous licorice extracts showed excellent cariostatic activity.

Ashit G. Bharwani and A. Suchetha. (2013)[54] evaluated the effect of Periocare® gum massage powder, which consists of Glycyrrhiza glabra, Rubia cordifolia, Piper nigrum, Cinnamon zeylanicum, and Eugenia caryophyllata, on reducing gingival inflammation. The results indicated that when gum massage powder was used in combination with mechanical therapy, microbiological and clinical improvements were observed.

Kalaiselvan Abinaya., Rajsekaran., Divya., and Jeyakumar. (2023)[55] evaluate the antimicrobial effect of licorice extracts and compare their action to commonly used root canal medicaments like calcium hydroxide and chlorhexidine against Enterococcus faecalis and Candida albicans. Ethanolic and aqueous extracts of licorice root were prepared. Antimicrobial activity was tested on E. faecalis and C. albicans using the Mueller-Hinton agar well diffusion method. Wells were prepared and filled with ethanolic extract, aqueous extract of liquorice, calcium hydroxide, and chlorhexidine. Samples were incubated at 37°C, and the zone of inhibition was examined after 24 h. They concluded that the ethanolic extract of licorice has a potent bactericidal effect against E. faecalis and C. albicans over the aqueous extract. Hence, it can be used as an intracanal medicament in routine endodontic therapy.

MATERIALS AND METHOD

The present study was conducted in the Department of Pediatric and Preventive Dentistry, Babu Banarasi Das College of Dental Sciences, B.B.D University Lucknow, India, after the approval by the Institutional Ethical Committee **(Annexure I-II)**, Babu Banarasi Das College of Dental Sciences, B.B.D University, Lucknow, India.

❖ STUDY DESIGN

This in-vivo randomized clinical trial was designed to evaluate the efficiency of two irrigation systems and the efficacy of different chemical and herbal irrigants.

❖ PARTICIPANTS

The study population consisted of systemically healthy children, aged 4-9 years, requiring endodontic treatment. All participants were selected randomly from the outpatient section, Department of Pediatric and Preventive Dentistry, Babu Banarasi Das College of Dental Sciences, B.B.D University, Lucknow, India.

❖ ELIGIBILITY CRITERIA

Children 4-9 years of age meeting the inclusion and exclusion criteria were included in the study after parental consent. **(Annexure III)**

❖ INCLUSION CRITERIA

1. Asymptomatic primary teeth with necrotic pulp.
2. Teeth with intact roots or less than 2/3rd of physiological root resorption.
3. Patients with no systemic conditions

❖ EXCLUSION CRITERIA

1. Teeth with excessive root resorption and mobility.
2. Children with special health care needs.
3. Patients who have received any antibiotics for three months before treatment.

SAMPLE SIZE CALCULATION

Healthy children with primary teeth will be included in the study. Sample size estimation was done by using GPower software (version 3.0). The sample size was estimated for ANOVA. A minimum sample size of 75 was sufficient for an alpha of 0.05, power of 80 %, and 0.57 as effect size (assessed from a similar study). Thus, the final sample size was further divided into 25 of each.

χ^2 tests - Goodness-of-fit tests: Contingency tables

Analysis: A priori: Compute required sample size

Input: Effect size w=0.4540017

α err prob=0.05

Power (1-β err prob) =0.95 Df=2

Output: Non-centrality parameter λ=15.4588158

Critical χ^2=5.9914645

Total sample size=75

Actual power =0.9501994

❖ RANDOMIZATION

Allocation to each group was done using three different coloured balls in a cardboard box, indicating each group. The box contained 75 balls in total. Three different colours for 25 balls each were allocated to a group. The participant was given to pick up a ball through the hole on top of the box. After the selection of a ball, it was removed from the box.

❖ INTERVENTION

Seventy-five samples of primary posterior teeth were irrigated using different irrigants.

Group I (n= 25)- 3% NaOCl irrigant (Figure 2)

Group II(n= 25)- 0.5% Chitosan irrigant (Figure 3)

Group III(n= 25)- 30%Liquorice irrigant (Figure 4)

ARMAMENTARIUM FOR PULPECTOMY PROCEDURE

Sr.No	Material & Armamentarium	Manufacturer
1	Single-sided mirror	MH3, GDC, India
2	Explorer	EXD5, GDC, India
3	Tweezer	DPU17, GDC, India
4	Spoon excavator	EXC133/134, GDC, India
5	Round diamond point	EX 24:Mani,Inc., Tochigi, Japan
6	Rubber dam kit	Hygenic, Coltene,Switzerland
7	Air turbine	NSK: S-Max Pico B2, Japan
8	K-files ISO #10-25	Mani, Inc., Tochigi, Japan
9	Paper point (#30),4%	Meta-Biomed, Republic of Korea
10	Normal Saline (0.9%w/v NaCl)	Beryl Drugs Ltd., India
11	Disposable syringe of 5ml	Dispo Van, India

ARMAMENTARIUM FOR MICROBIAL ANALYSIS

No	Material & Armamentarium	nufacturer
	Sterile Tweezer	GDC, India
	Sterile Absorbent Paper Points (4%,#30)	Meta-Biomed, Republic of Korea
	Eppendorf tubes	Amanta, India

TEST IRRIGANT

r.No.	Material & Armamentarium	nufacturer
1	NaOCl 3%	Vishal dent. PVT ltd
2	LIQUORICE 25%	Aceton biomed ,India
3	Chitosan nanoparticles 0.5 Wt%, 500 ml	Nano wings, India

FOR MICROBIOLOGY

Sr.No.	**Material & Armamentarium**	**Manufacturer**
1	Vortex mixer	Eltek VM 301
2	Blood agar plates	Accumix, India
3	Inoculation Loops	Himedia ,India
4	Digital colony counter	ESICO, India

METHODOLOGY

❖ PREPARATION FOR PULPECTOMY PROCEDURE.

Complete Oral prophylaxis of the dentition was done, and after the parents' consent, the pulpectomy procedure was commenced. The tooth, rubber dam, and clamp surfaces were cleaned with 30% hydrogen peroxide, followed by swabbing with 5% iodine tincture. **(Figure 1)** . Lignocaine containing 1:80000 adrenaline (Lignox, Warren, Mumbai, India) was administered, and the tooth was isolated with a rubber dam. After the endodontic access opening of the primary molar, pre-sampling sampling was collected using a sterile paper point from the pulp chamber and the largest diameter canal (Distal/Palatal).

The sterile paper point was kept in contact for 1 minute, and then cautiously obtained sample S1 was placed in a sterile collection tube filled with sterile Saline in a 1.5-ml tube disinfected prior with isopropyl alcohol. The endodontic irrigation sequence was performed by irrigating with normal Saline, followed by pulp debridement. Final irrigation was performed with the study irrigant for 5 minutes, and another sample (S2) **(Figure.10)**was collected from the largest canal. Samples were then transferred to a storage box and transported immediately to the microbiological lab for analysis.

❖ PRE-OPERATIVE SAMPLE COLLECTION

The pre-operative sterile paper point sample (S1) was marked with working length. After endodontic access opening of the molar tooth**(Figure.5)**, a sterile paper point was used to obtain the pre-sample (S1) **(Figure.6)**from the pulp chamber and canal with the largest diameter (Distal /Palatal canal). It was kept in contact for 60 seconds and cautiously obtained sample was placed in sterile collecting filled with sterile saline solution 1.5 ml tube**(Figure.7)** which was prior disinfected with Isopropyl alcohol.

❖ POST-OPERATIVE SAMPLE COLLECTION

Chemo-mechanical preparation was completed at the same appointment in all the cases. Canal preparation was completed to the working length, with hand nickel-titanium K-files in a back-and-forth alternating rotation motion using circumferential technique up to ISO #30- #35 size file. The 25 teeth designated for each group were irrigated with one of the irrigants **(Figure.1)**.

After irrigation, the post-operative paper point samples (S2) **(figure 9)** were placed for 60 seconds and then deposited into a sterile saline solution in a 1.5 ml tube**(figure 10)**.Post-operative samples were transported to the microbiology lab for microbiological culture.

❖ **MICROBIOLOGICAL PROCESSING**

The per-operative (S1) and post-operative (S2) paper point samples placed in 10 ml of thioglycolate broth were vortexed in a vortex mixer **(Figure.10)** for 1 min. Serial dilution of the broth was done by transferring 10 ml in the first test tube and 9 ml in the other 9 test tubes. The method is based on the principle that when a material containing a microorganism is cultured, each viable organism will develop into a colony. Hence, the number of colonies appearing on the plates**(Figure.14)** can represent the sample's living organisms.

This process is known as serial dilution and is done under laminar airflow **(Figure.12)**. Then, it was autoclaved at 121°C/15 psi for 15 minutes**(Figure.11)**. The samples were transferred from the test tube to the blood agar media using the micro-pipettes. The sampling test tubes were cultured at 30°C in an incubated for 24 to 48 hours**(Figure11)**, and colony-forming units were estimated using a microbial colony counter **(Figure.15)**.

The observations were laid down using the formula:

$$\text{-CFU/ml} = \frac{\text{(number of colonies X dilution factor)}}{\text{volume of culture plate.}}$$

The results were tabulated on an Excel sheet and sent for statistical Analysis.

Armamentarium for pulpectomy procedure

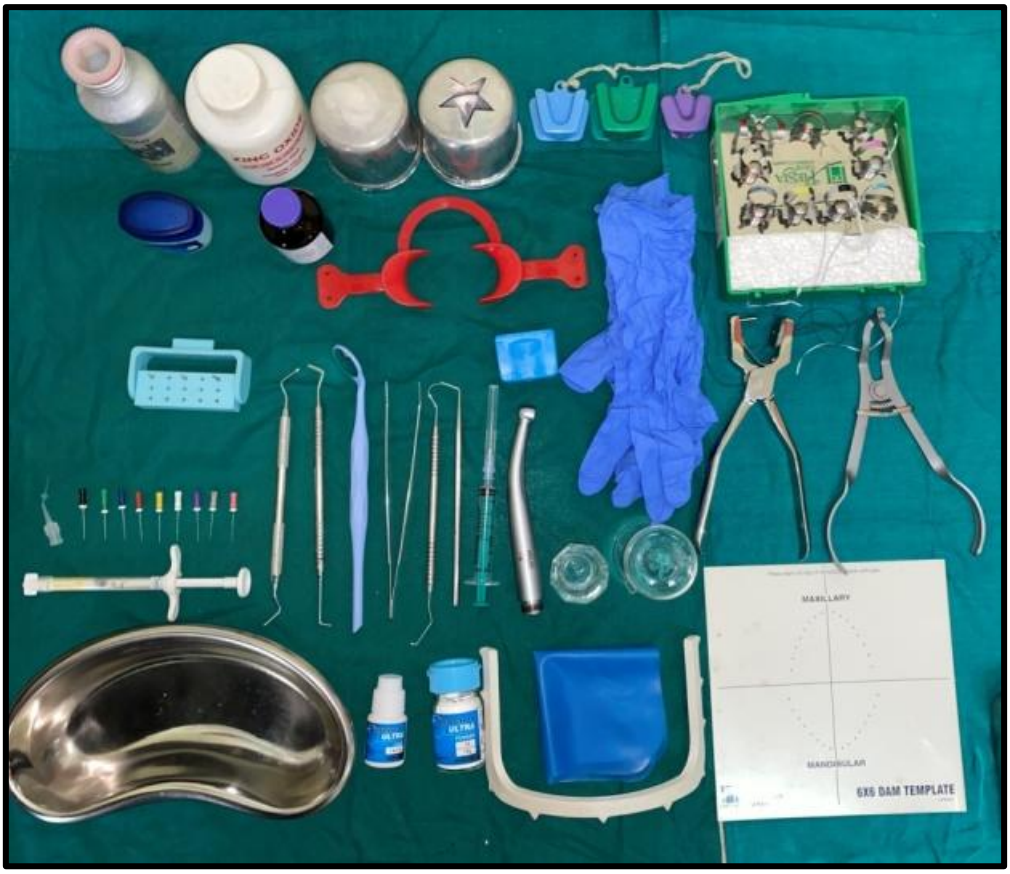

Figure 1.

Figure 2. 3% NaOCl

Figure3. 0.5% Chitosan.

Figure 4. 30% Liquorice

PRE-OPERATIVE PROCEDURE SAMPLE COLLECTION

. **Figure 5 Pre-Operative Endodontic access opening**

Figure 6. Pre-sampling with sterile paper point

Figure 7. Pre-Sampling S1

SAMPLE COLLECTION POST-OPERATIVE PROCEDURE

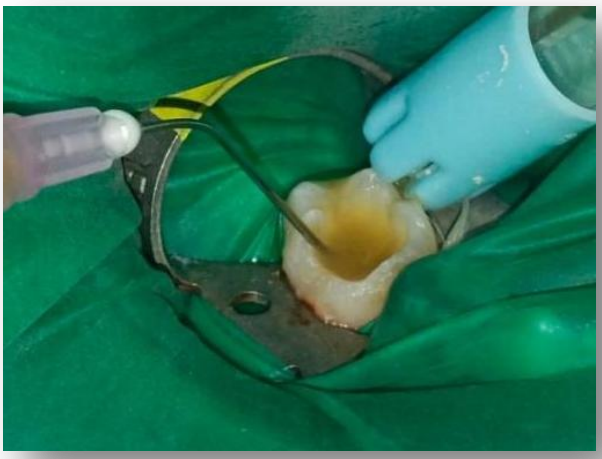

Figure8. Final irrigation with test irrigants.

Figure.9.PostSampling with sterile paper point .

Figure 10.Post sampling S2

Microbiological analysis procedure

Figure.11. Vortex mixer (Eltek VM 301)

Figure 12. Incubator .

Figure 13.Laminar flow.

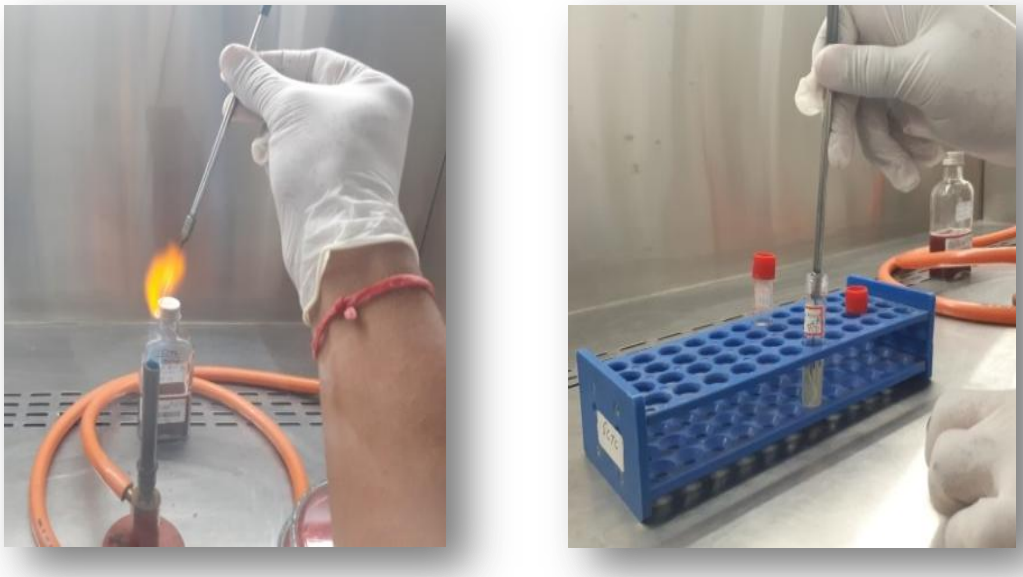

Figure 14.Inoculation procedure of Pre and post sampling and its processing

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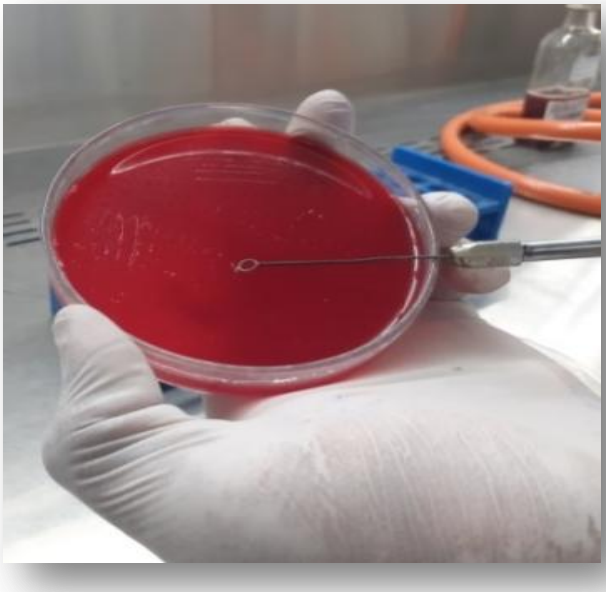

Figure.15.Blood Agar plate for microbial culture

Figure 16. Microbial growth seen on agar plate after 24-48hr.

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Figure 17.Digital Colony Counter.

OBSERVATION AND RESULT

A total of seventy-five primary molars were allocated according to inclusion criteria and were divided into three different groups (n = 25). Pre-operative and post-operative paper point samples were allocated and transported to the microbiological lab for processing. These samples of paper points were evaluated, microbial analysis was done, and CFU counts were noted on an excel sheet. These data were sent for statistical evaluation and are presented below.

❖ STATISTICAL ANALYSIS

The data for the present study was entered in Microsoft Excel 2007 and analyzed using the SPSS statistical software 23.0 Version. The descriptive statistics included mean, standard deviation frequency, and percentage. The level of significance for the present study was fixed at 5%.

The intragroup comparison will be done using the Paired t-test depending on the normality of the data. The SHAPIRO–WILK TEST was used to investigate the distribution of the data.

INTRAGROUP COMPARISON OF BACTERIAL COUNT FROM PRE TO POST TREATMENT LEVELS IN GROUP I(3% NaOCl)

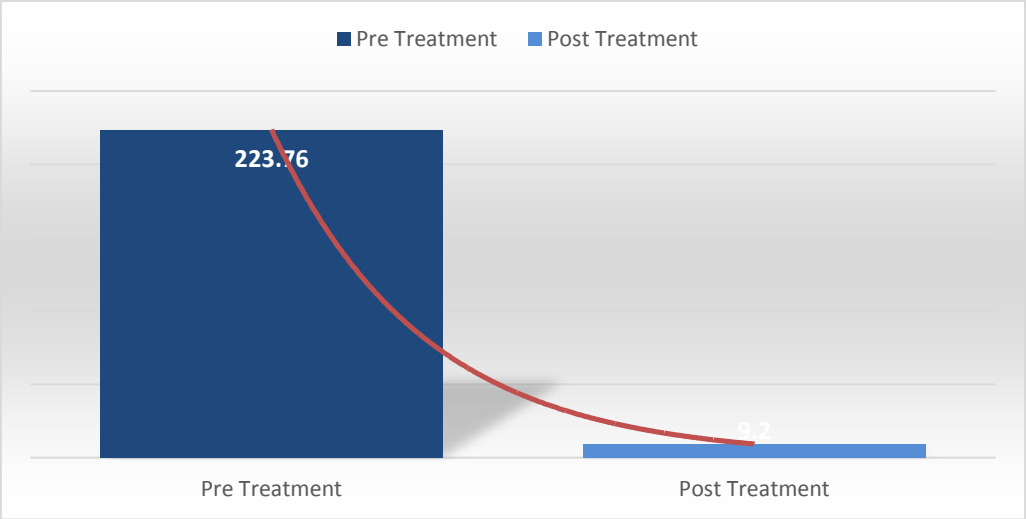
At the pre-treatment levels, the mean bacterial count in **Group I-3% NaOCl** was 223.76±69.95, and at the post-treatment level was 9.20±4.24. The mean change from the pre-treatment level to the post-treatment level was 214.56±70.51, and the percentage change from pre to post treatment-levels was 95.44±2.49.

Table 1. Intragroup comparison of bacterial count from pre to post-treatment levels in Group I-3% NaOCl

	Values	Mean Change	P value
Pre Treatment	223.76±69.95	214.56±70.51	0.001
Post Treatment	9.20±4.24		

The intragroup reduction in the bacterial count from pre to post-treatment levels was statistically significant in three groups when analyzed using a Paired t-test.

Graph 1. Intragroup comparison of bacterial count from pre to post treatment levels in Group I.



Above data is the graphical representation of comparison of bacterial count from pre to post treatment levels in Group I..The mean bacterial count in Group I was 223.76±69.95, and at the post-treatment level was 9.20±4.24. The mean change from the pre-treatment level to the post-treatment level was 214.56±70.51, and the percentage change from pre to post treatment-levels was 95.44±2.49.

Chart 1. Intragroup comparison of bacterial count from pre to post treatment levels in Group I-3% NaOCl

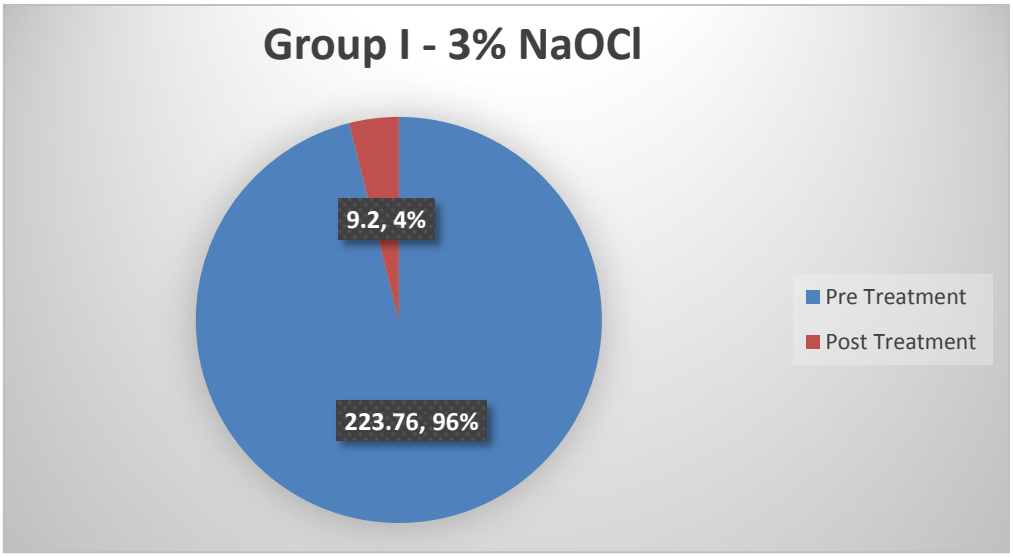


Chart 1 showing the intra-group comparison of mean bacterial count showing pre-treatments bacterial count was higher and on post treatments level , there has been a significant decrease in bacterial count . The mean bacterial count in Group I was 223.76±69.95, and at the post-treatment level was 9.20±4.24. The mean change from the pre-treatment level to the post-treatment level was 214.56±70.51, and the percentage change from pre to post treatment-levels was 95.44±2.49.

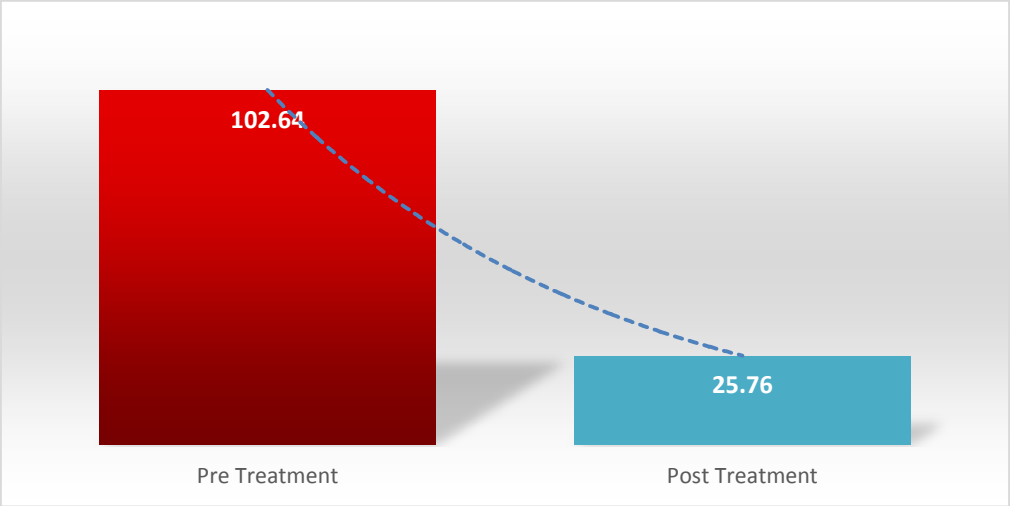
INTRAGROUP COMPARISON OF BACTERIAL COUNT FROM PRE TO POST TREATMENT LEVELS IN GROUP II (0.5% Chitosan)

In Group II, at the pre-treatment levels, the mean bacterial count was 119.12±55.12, and at the post-treatment level, it was 65.08±43.02. The mean change from the pre-treatment level to the post-treatment level was 54.04±26.66, and the percentage change from pre to post-levels was 47.44±17.12.

TABLE 2. Intragroup reduction in the bacterial count from pre to post treatment levels

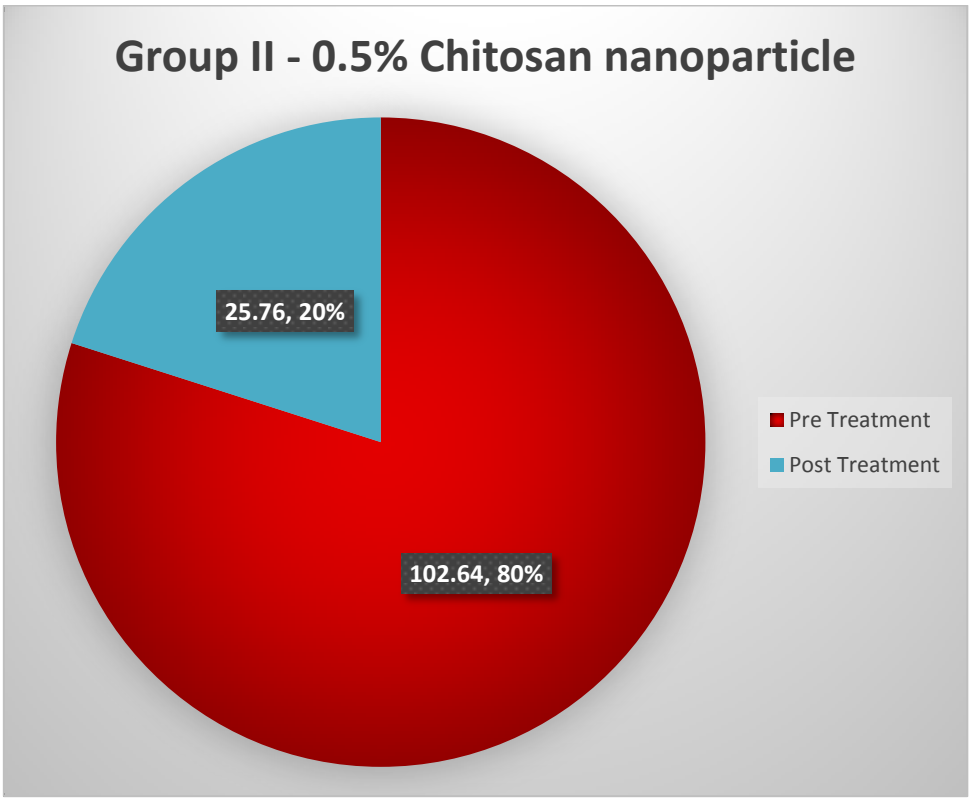
	Values	Mean Change	P value
Pre Treatment	102.64±44.40	76.88±32.75	0.001*
Post Treatment	25.76±14.63		

Graph 2..Intragroup reduction in the bacterial count from pre to post treatment levels in group II.



The intragroup reduction in the bacterial count from pre to post treatment levels was statistically significant in all three groups when analyzed using Paired t-test.

Chart 2.Intragroup reduction in the bacterial count from pre to post treatment levels in group II-0.5% Chitosan NP.



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Above pie chart represents the Intragroup reduction in the bacterial count from pre to post treatment levels in group II .In Group II, at the pre-treatment levels, the mean bacterial count was 119.12±55.12, and at the post-treatment level, it was 65.08±43.02. The mean change from the pre-treatment level to the post-treatment level was 54.04±26.66, and the percentage change from pre to post-levels was 47.44±17.12.

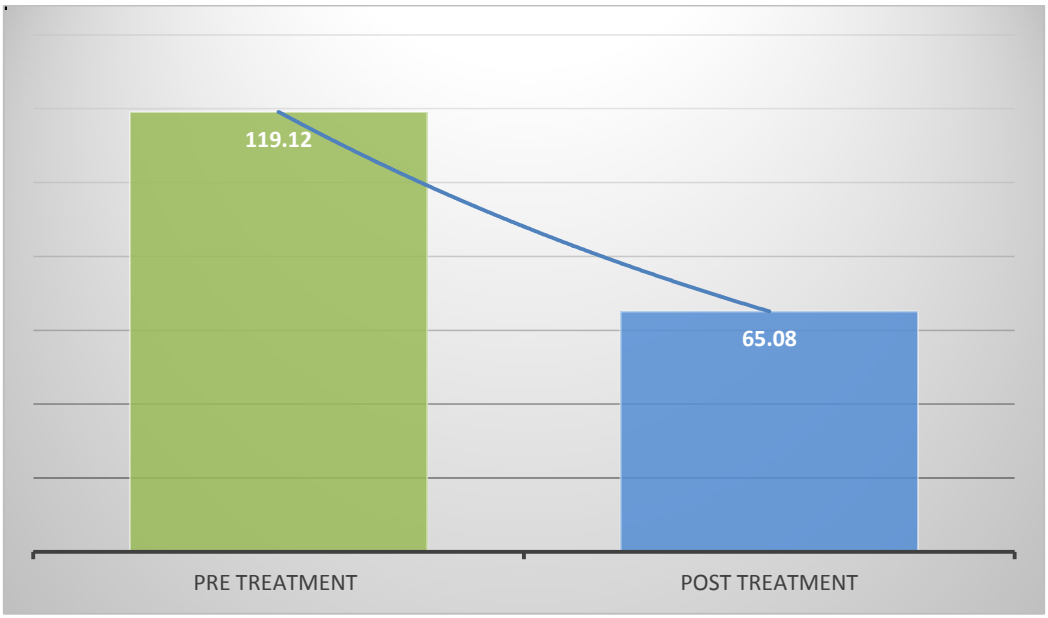
INTRAGROUP COMPARISON OF BACTERIAL COUNT FROM PRE TO POST TREATMENT LEVELS IN GROUP III (30%Liquorice)

In Group III, at the pre-treatment levels, the mean bacterial count was 102.64±44.40and, and at the post-treatment level, it was 25.76±14.63. The mean change from the pre-treatment level to the post-treatment level was 76.88±32.75, and the percentage change from pre to post-levels was 75.64±7.50.

Table.3.Intra-group reduction in the bacterial count from pre to post treatment levels in group III

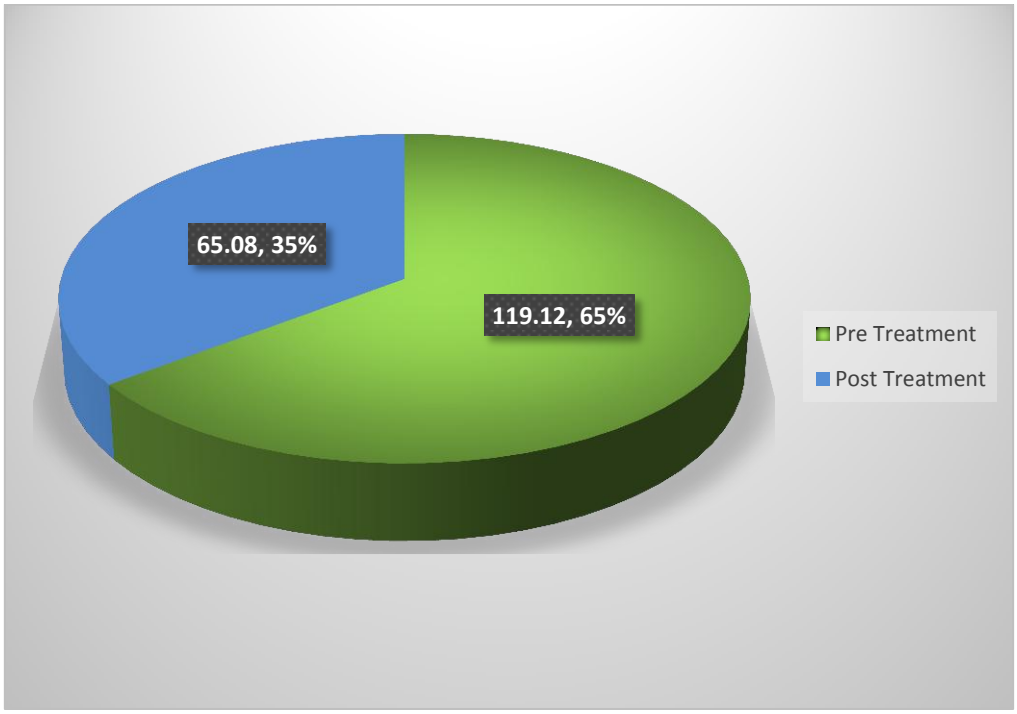
	Values	Mean Change	P value
Pre Treatment	119.12±55.12	54.04±26.66	0.001*
Post Treatment	65.08±43.02		

Graph 3. Intra-group reduction in the bacterial count from pre to post treatment levels in Group III-30% Liquorice



The intra-group reduction in the bacterial count from pre to post treatment levels was statistically significant in three groups when analyzed using Paired t test .

Chart 3. Intra-group reduction in the bacterial count from pre to post treatment levels in Group III



Above chart 3 is representation of intra group reduction in the bacterial count from pre treatment and post level in group III which is 30 % Liquorice group showing significant reduction in bacterial count analyzed on digital colony counter .The mean bacterial count was 102.64±44.40and, and at the post-treatment level, it was 25.76±14.63. The mean change from the pre-treatment level to the post-treatment level was 76.88±32.75, and the percentage change from pre to post-levels was 75.64±7.50.

Intergroup comparison of the change in bacterial count from pre to post-treatment levels between three groups

The intergroup comparison will be made using the ONE WAY ANOVA/KRSUSKAL WALLIS test followed by POST-HOC analysis depending upon the normality of the data.

Table.4 .Intergroup comparison of the change in bacterial count from pre to post-treatment levels between three groups

	Pre-Treatment	Post-Treatment	Mean Change	Percentage Change	P value
Group I	223.76±69.95	9.20±4.24	214.56±70.51	95.44±2.49	0.001*
Group II	102.64±44.40	25.76±14.63	76.88±32.75	75.64±7.50	
Group III	119.12±55.12	65.08±43.02	54.04±26.66	47.44±17.12	

At the pre-treatment levels, the mean bacterial count in Group I was 223.76±69.95, and at the post-treatment level was 9.20±4.24. The mean change from the pre-treatment level to the post-treatment level was 214.56±70.51, and the percentage change from pre to post-levels was 95.44±2.49.

Table 5. One-Way ANOVA Comparison.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	29089.142	2	14544.571	122.658	0.001*
Within Groups	8537.614	72	118.578		
Total	37626.756	74			

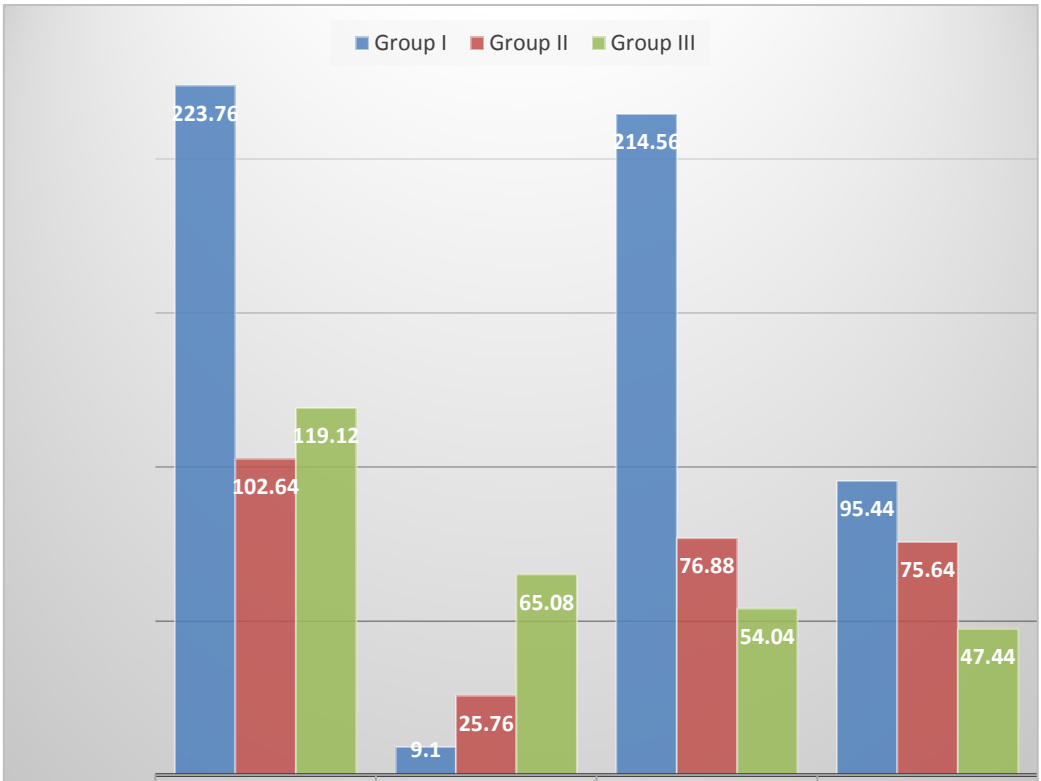
In Group II, at the pre-treatment levels, the mean bacterial count was 102.64±44.40and, and at the post-treatment level, it was 25.76±14.63. The mean change from the pre-treatment level to the post-treatment level was 76.88±32.75, and the percentage change from pre to post-levels was 75.64±7.50 .

Table 6.Post Hoc Analysis

Inter Group Comparison		Mean Change	Std Error	P value
Group I	Group II	19.80000*	3.07997	0.001 *
Group I	Group III	47.99617*	3.07997	0.001 *
Group II	Group III	-28.19618*	3.07997	0.001*

In Group III, at the pre-treatment levels, the mean bacterial count was 119.12±55.12, and at the post-treatment level, it was 65.08±43.02. The mean change from the pre-treatment level to the post-treatment level was 54.04±26.66, and the percentage change from pre to post-levels was 47.44±17.12

Graph 4. Intergroup comparison of change in bacterial count from pre to post treatment levels

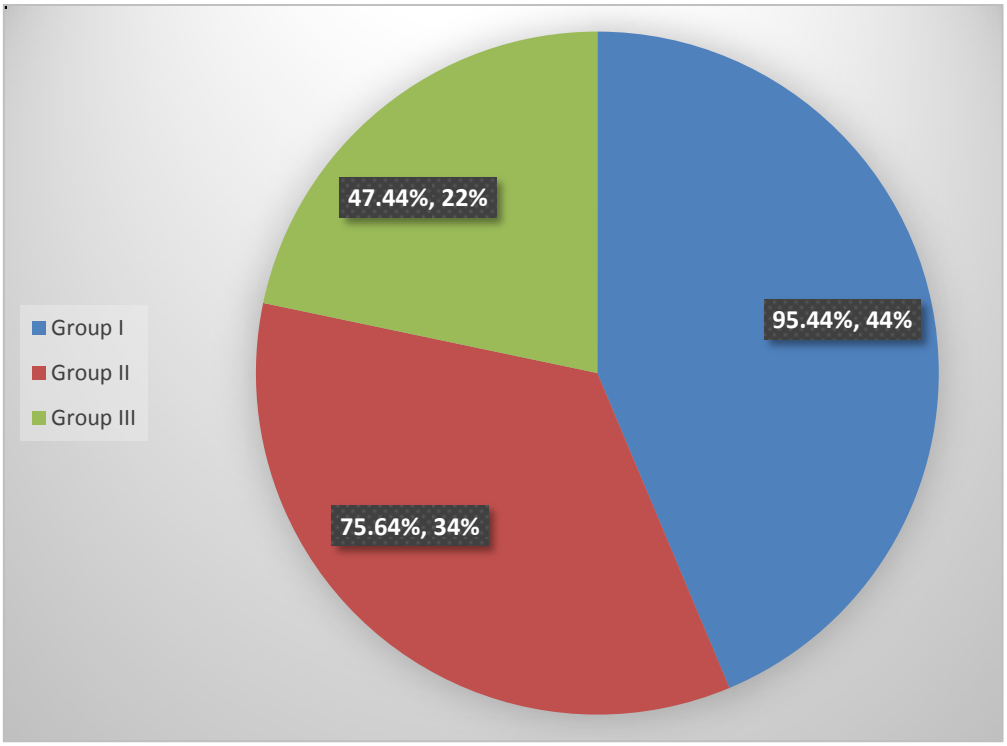


	Pre Treatment	Post Treatment	Mean Change	Percentage Change
Group I	223.76	9.1	214.56	95.44
Group II	102.64	25.76	76.88	75.64
Group III	119.12	65.08	54.04	47.44

The above graphical representing inter group comparison of change in bacterial count from pre treatment level to post treatment levels and there was significant reduction in all three groups.

The intergroup comparison of the change in bacterial count from pre to post-treatment levels was statistically significant between three groups with a sequence of change as follows: Group I>Group II>Group III.

Chart 4..Inter-group comparison of change in bacterial count between three different groups.



Above Pie chart is therepresentation of intergroup comparison in bacterial count analyzed on digital colony counter .At the pre-treatment levels, the mean bacterial count in Group I was 223.76±69.95, and at the post-treatment level was 9.20±4.24. The mean change from the pre-treatment level to the post-treatment level was 214.56±70.51, and the percentage change from pre to post-levels was 95.44±2.49.In Group II, at the pre-treatment levels, the mean bacterial count was 102.64±44.40and, and at the post-treatment level, it was 25.76±14.63. In Group III, at the pre-treatment levels, the mean bacterial count was 119.12±55.12, and at the post-treatment level, it was 65.08±43.02.

The intergroup comparison of the change in bacterial count from pre to post-treatment levels was statistically significant between three groups with a sequence of change as follows: Group I>Group II>Group III.

DISCUSSION

In recent years, there has been a significant change in how root canal treatment is performed on human primary teeth[56]. As we know, relying solely on mechanical instrumentation will not be sufficient in removal of the microbes from the root canal due to the varied nature of endodontic infections and the variations in tooth anatomy[57]. Since the root canals of primary teeth have a complex anatomy, it is challenging to access certain areas such as isthmi, canal fins, cul-de-sacs, lateral canals, and accessory canals[58].

Using endodontic irrigants during root canal therapy helps dissolve and remove any remaining debris that could not be reached or removed with instruments alone. Endodontic irrigation ensures the complex root canals of teeth are thoroughly cleared and disinfected, which is crucial for the procedure's success. Various irrigating solutions have been attributed for use in the evidence based literature of endodontics for irrigation purposes, out of which sodium hypochlorite is most commonly used[59,60].

Sodium hypochlorite is a widely used chemical irrigant in dentistry and is considered the gold standard irrigant. It demonstrates a potent antimicrobial activity against the microbiota of infected root canals. NaOCl mechanism of action as it ionizes in water into Na+, and the hypochlorite ion, OCl-, establishes equilibrium with hypochlorous acid (HOCl). Chlorine exists predominantly as HOCl at acidic and neutral pH, whereas OCl- predominates at pH of 9 and above. Hypochloric acid is responsible for the antibacterial activity[61].

Chitosan is a natural polysaccharide obtained by chitin deacetylation, one of the most abundant natural polysaccharides found in the exoskeleton of shrimps and crabs. It is one of the most researched polymeric nanoparticle materials in endodontics. A modification in the chemical structure of chitin (1,4)-N-acetyl-D-glucos-2-amine leads to the formation of (1,4)-2-Amino-2-desoxy- beta-D-glucan, known as Chitosan. Chitosan is biocompatible, biodegradable, bioadhesive, and has no reported toxicity. Besides, it is an excellent antimicrobial agent, and its low production costs have increased its utility for various applications in medicine and pharmaceuticals. In

addition, it has a high chelating capacity for different metal ions in acidic conditions[62].

Chitosan antibacterial mechanism is still under research. One hypothesis states that Chitosan can bind with the negatively charged cell membrane, which enhances its permeability, leading to the cytoplasm and its content leakage and, ultimately, bacterial cell death. Other hypotheses state that Chitosan has the property to chelate metal, reducing enzyme activity and inhibiting bacterial action.[63]

Liquorice is an ancient herb used in Ayurvedic medicine that has Glycyrrhizin and Glabridin compounds. It protects mitochondria, prevents LDL oxidation, and has antimicrobial properties[64]. Saponins exhibit antibacterial action[65], while flavonoids inhibit bacterial cells' oxygen consumption[66] and respiratory electron transport[67]. Liquorice is effective against polymicrobial species and has been found to be biocompatible with fibroblast cells[68]. Studies have shown that liquorice has antimicrobial and anti-tubercular properties, and it is used to treat various health conditions[69]. Flavonoids present in liquorice extract have antibacterial and anti-Helicobacter pylori activities[70].

There are limited comphrensive *in-vivo* literature in dentistry regarding the efficacy of Chitosan nanoparticles and Liquorice in different clinical scenarios. The current *in-vivo* literature must provide more evidence or supporting clinical data to determine the practical application of both irrigating solutions. However, in a few *ex-vivo* studies, Chitosan and Liquorice solutions have been found to have antimicrobial and therapeutic effects and may be used as an endodontic irrigant.

Therefore, this *in-vivo* study was designed to compare three irrigating solutions using chemical, bio-material, and herbal irrigation solutions on primary tooth root canals. The present study was conducted in the Pediatric and Preventive Dentistry Department, "Babu Banarasi Das College of Dental Sciences", B.B.D. University, Lucknow, India. Before enrolling the children in the study, we obtained clearance from the Institutional Ethical Committee **(Annexure I and Annexure II)**, as well as written informed consent from their parents **(Annexure III)**. The objective was -

1. To evaluate the antimicrobial efficacy of Sodium hypochlorite, Chitosan, and Liquorice as root canal irrigants in the primary teeth.

2. To compare the antimicrobial activity of Sodium hypochlorite, Chitosan, and Liquorice as root canal irrigants in the primary teeth.

Infections in endodontic, are characterized by poly-microbial and with obligate anaerobic bacteria being the dominant microbiota of primary infections. Various microorganisms are related to intra-radicular and extra-radicular infections and persistent infections. Enterococcus faecalis are widely chosen for *ex-vivo* endodontics studies due to their resistance to conventional endodontic therapies, virulence factors, and ability to out-compete other bacteria. Hence, our study aimed at poly-microbial species and the antimicrobial efficacy of three different irrigants.

Various methods have been suggested for collecting microbial samples from the root canals, including using paper points and collecting dentinal shavings from the internal root surface using files. Paper point cultures of the root canals detected bacteria more frequently than dentin filing cultures on files or reamers. In the current studies, we used a paper point for the sampling collection procedure, which was not technique-sensitive and could be sterilized in an autoclave[71].

The teeth selected for sample collection were the primary molar and the canal from which the microbial sample was collected from the distal or palatal canal, which is the widest diameter canal.

Our *in-vivo* randomized clinical trial was designed to evaluate the antimicrobial efficaccy of three irrigation solutions: **Group I - 3% NaOCl**, **Group II - 0.5% Chitosan**, and **Group III - 30%Liquorice** .

In the present study, **Group I - 3%Sodium Hypochlorite(NaOCl)** showed the highest mean percentage reduction in the bacterial count the percentage change from pre to post-levels which was 95.44±2.49, shown in **Graph.1**. The difference was statistically significant

in **Table.1** . These results are in agreement with the findings of a study conducted by **Golob et al.(2017)**[72] who found that effective decontamination of the

root canals can only be achieved by applying NaOCl at a high concentration (3%NaOCl), as bacteria tend to regrow 48 hours after being treated with 1% and 2% NaOCl. Further, their study revealed that 1% NaOCl partially reduced bacterial vitality in the biofilm, while the 2.5% and 3.% NaOCl solutions caused complete inhibition of the biofilm bacterial growth[73]. Similarly, **Clegg et al.(2028)[74]** found that 3 % and 6% NaOCl showed the absence of biofilm, and 1 % NaOCl showed biofilm disruption. Likewise, a study conducted by **Dumitriu and Dobre et al.(2015)[**[75] found the concentration of 3% NaOCl positively correlated with the rate of collagen dissolution. **Swimberghe et al.(2021)[**[76] reported significantly different biofilm-eradication effectiveness between NaOCl concentrations at 0.025, 0.1, 0.5,2.5%, and 3% for anaerobically and aerobically incubated E. faecalis biofilms which are similar to our study results.

As seen in our study result, **Group I** showed a maximum reduction in the bacterial cooi unt as 214.56±70.51 in post-irrigation, observed in **Chart I.** The reason for such a significant reduction is explained in a study by **Aranda-Garcia AR et al.[77]**, who found that 3% Sodium hypochlorite helps remove vital pulp tissues from dentinal walls. All forms of chlorine in NaOCl solution, including hypochlorous acid (HOCl) and hypochlorite ion (OCl-), are collectively referred to as "free available chlorine"[78] By direct contact with the organic matter, the free available chlorine molecules lead to amino acid degradation and hydrolysis, manifesting as tissue dissolution[79]. **Stojicic et al.(2010)[80]** reported that the tissue breakdown increased almost proportionally with the concentration, and the antibacterial efficacy is directly related to its concentration. The higher concentrations (3%-5%) are more effective than the mild concentrations(2%-2.5%) and lower concentrations (1-1.5%)[81,82].

Various concentrations of sodium hypochlorite have been used for endodontic irrigation. There are several significant disadvantages associated with the use of sodium hypochlorite. These include its irritant effect on periapical tissues, high toxicity, tendency to corrode instruments, unpleasant taste, and inability to remove the smear layer effectively[83,84]. Moreover, there is a risk of extrusion into periapical tissues that results in inflammation, hematoma, necrosis, paresthesia, and harm to permanent tooth buds[85,86].

Therefore, the concentration of sodium hypochlorite was limited to 3% to minimize the risk of post-treatment periapical tissue irritation and adverse effects over permanent buds.

In the present study, **Group II - 0.5%Chitosan nanoparticle** ,showed the mean change from the pre-treatment level to the post-treatment level was 54.04±26.66, and the percentage change from pre to post-levels was 47.44±17.12 showed the highest mean percentage reduction in the bacterial count**(Graph.2)**. The result of present study was in accordance of study conducted by **Kishen et al. (2008). & Shrestha et al. (2010)[87]**, who evaluated the efficacy of Chitosan as an irrigant against E. Faecalis in a biofilm & planktonic state. They concluded that Chitosan is effective in completely eliminating the planktonic state and significantly reducing the biofilm state. **Del Caprio-Peeochena A et al. (2015)[88]** evaluated the efficacy of nano-chitosan in removing the smear layer and inhibiting bacterial growth in dentin. They concluded that nano-chitosan could eliminate the smear layer and prevent bacterial colonization in dentin. Thus, it can be used as a final irrigant and as an alternative to EDTA during root canal treatment.

Similarly, the study conducted by **Chaudhari DV et al.(2020)**[89], the antimicrobial efficacy of Chitosan Nanoparticles (C.N.P.s), Silver Diamine Fluoride (S.D.F.), and Bioactive Glass Nanoparticles (BAGNP) as root canal irrigants against the bacterial strain of Enterococcus Faecalis was evaluated. The results showed the most significant zone of inhibition in Chitosan, followed by S.D.F. and Bioactive Glass Nanoparticles[90].

In the present study, the **Group III - 30%Liquorice** showed the percentage change in bacterial count from pre to post treatment levels was 47.44±17.12. The antibacterial efficacy was significantly lower ,as shown in **Table.3.** A study by **S Rekha et al.(2015)[91]** is in accordance to our study . They described the antimicrobial efficacy of three herbal irrigants: Propolis, Liquorice, German chamomile and chemical irritants like 5% Sodium hypochlorite (NaOCl) against root canal microbes. Their findings agree with the conclusion that Sodium hypochlorite was the most effective antimicrobial agent as an endodontic irrigant. Propolis, Liquorice, and

German chamomile were also effective, but less in comparison than sodium hypochlorite, The present findings contrast with the findings of **Sumit Rajewar et al.(2020)**[92], who found Liquorice more effective than sodium hypochlorite in reducing the bacterial load assessed by a zone of inhibition.

Gupta V.K. et al. (2008)[93] stated that aqueous and ethanolic liquorice extracts are potent cariostatic agents and are palatable in child patients. **Salehi M (2018)**[94] stated that Liquorice effectively reduces pain and ulcers in stomatitis.**Badr et al.** conducted a study comparing the biocompatibility of Liquorice and Ca(OH)2 with fibroblast cells. They found Liquorice effective against E. faecalis when used as an intracanal medicament[95].The result of this study are in accordance with our study (**Table. 3**)and observed in the intra-group comparison of **Group III**.

The present study's findings contrast the findings of **Shenoi PR et al. (2016)**[96], who found that there was statistically no significant difference between the antibacterial efficacy of 0.5% chitosan and 3% sodium Hypochlorite. The result of present study found a significant difference in bacterial pre and post level in compariso of **Group II (Graph 1)** and **Group I (Graph 2)**.

A study by **Silva et al.(2013)**[97] observed the antibacterial activity of Chitosan. It stated that Chitosan had a practical antimicrobial effect on the root canals after instrumentation, which are in accordance to our present study **(Table 2)**. Also, Chitosan attaches to D.N.A. and inhibits mRNA synthesis by passing to the microorganism's nuclei and interfering with mRNA and protein synthesis[98].

The results are in agreement with the findings of an inter-group comparison of a study conducted by **Asmaa M. Faiek et al. (2019)**[99], where Chitosan when compared with 3%NaOCl, showed that the NaOCl group showed statistically significantly highest mean percentage reduction in Enterococcus faecalis counts and the Chitosan group showed statistically significantly lower mean value than 3%NaOCl.

The present study, **Group II - 0.5% Chitosan N.P**., showed the mean bacterial count as 119.12±55.12; at the post-treatment level, it was 65.08±43.02.In the current

study, **0.5% Chitosan nanoparticle** showed significant antibacterial activity but was lower than sodium hypochlorite.

However, in the study conducted by **Pallavi Goel et al.(2022)**[100], the intergroup comparison between the efficacy of 0.2% chitosan and 3% sodium hypochlorite as an antibacterial agent showed Chitosan to be more effective than sodium hypochlorite in reducing the bacterial count in root canals , which was not in accordance to the findings of our study in intergroup in **Table 6**. The present study findings also agree with the findings of **Nagarjuna et al. (2017)**[101]., who reported the efficacy of 3% sodium hypochlorite to be highest compared to Chlorhexidine and Chitosan.

Yadav et al.[102] conducted a study to evaluate the antibacterial efficacy of 3% NaOCl and 0.5% Chitosan on E. faecalis and Candida Albicans. The study results indicated that 3% NaOCl had the lowest colony-forming units (CFU=32/ml), followed by 0.5% chitosan (CFU=33/ml), but the findings were not significant ,which is in contrast to the result of our present study ,as shown in **Table 6**.

For evaluation of the antimicrobial activity of irrigants in *ex-vivo* studies, the colony-forming unit(CFU) method is the gold standard. Therefore, this method was adopted in this study. Laboratory and clinical setup errors limit *ex-vivo* studies. Contamination of the irrigants with other biofilms and fluids in the oral cavity may alter the results. The root canal microorganisms other than E. faecalis may react unpredictably to the studied irrigants. Agar depth can affect the accuracy of plate-based assays, and the probable reason for this could be antimicrobial agent diffusion. Therefore, further clinical studies are indicated.

From the results of the study, the following conclusions can be arrived that 3% Sodium hypochlorite was found to be the most effective antimicrobial agent as an endodontic irrigant and showed a statistical significance difference in the antimicrobial activity between 3% Sodium hypochlorite and Chitosan. The antimicrobial activity of Liquorice was significantly less when compared to 3% Sodium hypochlorite and Chitosan. Concerning the adverse reactions of chemical irrigants, Chitosan, a Biomaterial, may be considered as an alternative to chemical irrigants.

Long-term clinical trials may be required to evaluate the properties of Chitosan nanoparticles and Liquorice before these may be conclusively or practically recommended as an intracanal irrigating solution as an alternative to sodium hypochlorite.

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CONCLUSION

The present *in-vivo* study evaluated & compared antimicrobial efficacy of 3% Sodium hypochlorite ,0.5% Chitosan nanoparticles and 30 % Liquorice.

Within the limitations of this study the following conclusions were drawn:

1. 3% Sodium hypochlorite was found to be the most effective antimicrobial agent as an endodontic irrigant and showed a statistical significance difference in the antimicrobial activity between 0.5%Chitosan and 30% Liquorice .

2. The antimicrobial activity of 0.5% Chitosan nanoparticle was significantly better when compared to 30% Liquorice.

3. Considering the adverse reactions of chemical irrigants,, a Biomaterial : Chitosan, or Herbal irrigant-Liquorice may be considered as an alternative to chemical irrigants

Long-term clinical trials may be required to evaluate the properties of Chitosan nanoparticles and Liquorice before these may be conclusively recommended as an intracanal irrigating solution as an alternative to sodium hypochlorite.

SUMMARY

Primary teeth are essential for harmonious occlusion growth, arch length preservation, and functional activity like chewing and speech in children. Endodontic infections are caused by opportunistic microorganisms, which invade the deciduous tooth root canals containing necrotic tissue and begin an infectious process. Eliminating these microorganisms from the root canal system should be the objective of root canal treatment. A pulpectomy is a non-vital pulp therapy for primary teeth. It involves removing infected pulp tissue, debris, and microorganisms with an irrigant and obturating the canals with an appropriate material.

Using endodontic irrigants during root canal therapy helps dissolve and remove any remaining debris that could not be reached or removed with instruments alone. Endodontic irrigation ensures the root canals are thoroughly cleaned and disinfected, which is crucial for the procedure's success.Various irrigating solutions have been attributed for use in the field of endodontics for irrigation purposes, out of which sodium hypochlorite is most commonly used.

The present study was conducted in the Pediatric and Preventive Dentistry Department, Babu Banarasi Das College of Dental Sciences, B.B.D. University, Lucknow, India. Clearance was taken from the Institutional Ethical Committee, and written informed consent was obtained from parents before the enrolment of the children in the study. The objective was -

1. .To evaluate the antimicrobial efficacy of sodium hypochlorite, Chitosan and Liquorice as root canal irrigants in primary teeth.

2. To compare the antimicrobial activity of sodium hypochlorite, Chitosan and Liquorice as root canal irrigants in primary teeth

Seventy-five samples of primary posterior teeth were irrigated using different irrigants.

Group I (n= 25)- 3% NaOCl irrigant

Group II(n= 25)- 0.5% Chitosan irrigant

Group III(n= 25)- 30%Liquorice irrigant

Complete Oral prophylaxis of the dentition was done, and after the parents' consent, the pulpectomy procedure was commenced. The tooth, rubber dam, and clamp surfaces were cleaned with 30% hydrogen peroxide, followed by swabbing with 5% iodine tincture . Lignocaine containing 1:80000 adrenaline (Lignox, Warren, Mumbai, India) was administered, and the tooth was isolated with a rubber dam. After the endodontic access opening of the primary molar, pre-sampling sampling was collected using a sterile paper point from the pulp chamber and the largest diameter canal (Distal/Palatal).

The sterile paper point was kept in contact for 1 minute, and then cautiously obtained sample S1 was placed in a sterile collection tube filled with sterile Saline in a 1.5-ml tube disinfected prior with isopropyl alcohol. The endodontic irrigation sequence was performed by irrigating with normal Saline, followed by pulp debridement. Final irrigation was performed with the study irrigant for 5 minutes, and another sample (S2) was collected from the largest canal. Samples were then transferred to a storage box and transported immediately to the microbiological lab for analysis.

From the results of the study, the following conclusions can be arrived that 3% Sodium hypochlorite was found to be the most effective antimicrobial agent as an endodontic irrigant and showed a statistical significant difference in the antimicrobial activity between 3% Sodium hypochlorite and Chitosan. The antimicrobial activity of Liquorice was significantly less when compared to 3% Sodium hypochlorite and Chitosan.

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ANNEXURES

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