Evaluation of efficacy of sodium hypochlorite, triple antibiotic, MTAD, and garlic extract root canal irrigants in endodontically treated teeth

Running title: Efficacy of Root canal irrigants

Authors

1. Dr. Swati Budhiraja
Lecturer, Department of Conservative Dentistry and Endodontics
MM Institute of Dental Sciences and Research (MM Deemed to be University),
Ambala, Haryana, India

2. Dr. Laxmikant Late
Assistant Professor, Department of Conservative Dentistry & Endodontics
Dr. DY Patil Dental College and Hospital
Dr. DY Patil Vidyapeeth, Pune, Maharashtra, India

3. Dr. Atul Bajoria
Lecturer, Department of Oral Medicine and Radiology
Kalinga Institute of Dental Sciences
KIIT University Patia, Bhubaneswar 24, Odisha, India

4. Dr. Kapil Kamal Kishore Fafat
Reader, Department of Orthodontics and Dentofacial Orthopedic
Dr. Rajesh Ramdaji Kambe Dental College and Hospital
Akola, Maharashtra, India

5. Dr. Prabu Mahin Syed Ismail
Department of Restorative Dentistry
College of Dentistry in Al-Rass
Qassim University, Kingdom of Saudi Arabia

6. Dr. Anupa Samanta
Tutor, Department of Oral Medicine and Radiology
Kalinga Institute of Dental Sciences
KIIT University, Bhubaneswar 24, Odisha, India

Corresponding author

Dr. Atul Bajoria
Lecturer, Department of Oral Medicine and Radiology
KAlinga Institute of Dental Sciences
KIIT University Patia, Bhubaneswar 24, Odissa, India
E-mail: atultink@gmail.com
Abstract

Introduction: Successful root canal treatment involves complete removal of all vital or necrotic tissue, micro-organisms, and its products from the root canal system.

Objectives: To evaluate the antimicrobial efficacy of sodium hypochlorite, triple antibiotic, MTAD, and garlic extract root canal irrigants in endodontically treated teeth.

Materials and method: Sixty samples were categorized into four groups with 15 samples in each for intracanal irrigation; Group I: 3% NaOCl, Group II: Triple antibiotic, Group III: MTAD, Group IV: garlic extract. E. faecalis ATCC 29212 was used in the study. In brain heart infusion (BHI) agar plates, each medicament was added to the respective wells. The plates were incubated in an incubator at 37°C for 24 h. After incubation, plates were removed and the bacterial inhibition zone around each well was noted. Results were statistically evaluated with SPSS software for Windows version 21.0. (IBM Corp., Armonk, NY) with analysis of variance (ANOVA) tests (P < 0.001).

Results: Sodium hypochlorite (32.45) showed highest mean inhibitory zone with highest antibacterial efficacy falledow by Triple antibiotic paste irrigant (29.32), MTAD (22.45) and least by herbal product garlic extract (19.89).

Conclusion: The tested irrigants, Triple antibiotic paste and MTAD showed antibacterial efficacy comparable to Sodium hypochlorite. Hence these irrigants can be used in endodontic procedure.

Key words

Antibacterial, efficacy, Irrigamt, MTAD, Root canal

Introduction

Successful root canal treatment involves the complete removal of all necrotic tissue, microorganism from the root canal and the three-dimensional obturation of the canal space.\(^1\)\(^-\)\(^3\) It is well known that microorganisms in the root canal are responsible for pulp and periradicular infections. Enterococcus faecalis is the most commonly found bacteria in failed root canal. It is an
anaerobic gram-positive bacterium responsible for 80–90% of enterococcal infection. It plays an essential role in persistent failure of endodontic therapy. Only with mechanical cleaning, all microorganisms cannot be eliminated. Chemical irrigation of canals along with biomechanical preparation helps in the elimination of microorganisms.¹

Sodium hypochlorite (NaOCl) and 2% chlorhexidine (CHX) are chemical root canals irrigants used successfully. CHX is a strong antibacterial and is effective against E. faecalis, which causes endodontic failure. It is bacteriostatic in low concentration and bacteriocidal in high concentration.¹ Sodium hypochlorite is the most commonly used irrigant because it fulfills most of the requirements as an ideal endodontic irrigant.⁴

Ideal root canal irrigants should be biocompatible, nontoxic, with a desirable smell and taste. Chemical irrigants, even though effective in root canal irrigation, are associated with several disadvantages. NaOCl causes allergic reaction, tissue toxicity, staining of instruments, irritation to periapical tissue, inability to remove smear layer, and has an undesirable smell and taste. CHX when mixed with sodium hypochlorite produces a carcinogenic product, i.e., parachloroanaline. CHX has disadvantages of undesirable smell and taste as well as tissue toxicity.¹ Hence alternative effective and safe intra canal irrigants were tested.

Various alternative root canal irrigants were tried such as antibiotic paste, herbal products, and MTAD.¹,⁵,⁶,⁷

In 1928 antibiotics were first concealed, even though in 1951 Grossman first reported local use of an antibiotic in endodontics. In dentistry including endodontics, antibiotics may be administered systemically and applied locally as an endodontic irrigant or endodontic medicament. Various antibiotics have been used which includes tetracycline, metronidazole, ornidazole, ciprofloxacin and minocycline.⁵

MTAD has been introduced to dentistry as a final irrigant for elimination of the smear layer. MTAD (BioPure, DENTSPLY, (Tulsa Dental, JohnsonCity, TN) is the irrigating solution of the mixture of tetracycline isomer (doxycycline), an acid (citric acid), and a detergent (sodium lauryl sulfate/Tween-80).⁷ BioPure MTAD is less toxic than NaOCL. This solution contains 3% doxycycline hyclate which helps to remove smear layer, tetracycline for sustainability, 4.25%
citric acid which acts as a chelating agent and a detergent 0.5% polysorbate 80 which reduces the surface tension and increases its wettability. 6

Various natural plant extracts have antimicrobial properties and therapeutic effects suggesting their potential to be used as an endodontic irrigant such as garlic extract, neem, turmeric, aloe vera, green tea extract and honey which have an antimicrobial, anti-inflammatory & antioxidant properties.4,8 Garlic (Allium sativum) is bulbous perennial vegetable spice belongs to family Alliaceae with antibacterial, antifungal and antiviral properties. So, it can be used as endodontic irrigant.9

The present study was aimed to evaluate the antimicrobial efficacy of Sodium hypochlorite, triple antibiotic, MTAD, and garlic extract root canal irrigants in endodontically treated teeth.

Materials and Methods

This in vitro study was done in the department of conservative Dentistry and Endodontics. Total 60 samples were divided into 4 groups with 15 samples in each group for evaluation of inhibition zone around E faecalis. Group I: 3% NaOCl, Group II: Triple antibiotic, Group III: MTAD, Group IV: garlic extract.

Preparation

3 % Sodium hypochlorite: Commercially available solution was used for the study

MTAD: BioPure MTAD (Dentsply, Tulsa Dental, Tulsa, OK, USA) was used for the study.

Preparation of Triple Antibiotic Solution: All the raw ingredients were weighed with the help of electronic balance as per the formulation sheet. Sucrose powder was sieved through the # 20 micron mesh and collected in a separate container. All other ingredients were also sieved through the mesh and collected in a separate container. After which the materials were mixed with sucrose powder and triturated with a glass mortar and pestle to evade formation of any lumps. In a polythene bag the mixture was collected and packed for further use.

Preparation of garlic extract - Freshly peeled cloves of garlic were shade dried and powdered. 5grams of garlic powder was macerated with 100 ml of distilled water. The homogenate was
filtered using Whatman’s filter paper no. 1. This extract was heated over water bath for 6 hours till it become slightly viscous.

**Microbiological analysis**

E. faecalis ATCC 29212 (HiMedia Laboratories Pvt. Ltd., Mumbai, India) was collected and grown overnight in brainheart infusion (BHI) broth at 37°C and inoculated in Mueller–Hinton agar plates and adjusted to 0.5 turbidity reading on McFarland scale (1.5 × 10^8 bacteria/ml). Once BHI agar plates were prepared, each medicament was added to the respective wells. The plates were incubated in an incubator at 37°C for 24 h. After incubation, plates were removed and the bacterial inhibition zone around each well was recorded. Results were tabulated and statistically evaluated using Statistical Package for the Social Sciences for Windows, version 21.0. (IBM Corp., Armonk, NY) with analysis of variance (ANOVA) tests (P < 0.001).

**Results**

Table 1 indicates inhibitory zone against Enterococcus Faecalis. Sodium hypochlorite (32.45) showed highest mean inhibitory zone with highest antibacterial efficacy fallowed by Triple antibiotic paste irrigant (29.32), MTAD (22.45) and least by herbal product garlic extract (19.89).

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>Sd</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hypochlorite</td>
<td>32.45</td>
<td>1.148</td>
<td>0.398</td>
</tr>
<tr>
<td>Triple antibiotic</td>
<td>29.32</td>
<td>1.123</td>
<td>0.315</td>
</tr>
<tr>
<td>MTAD</td>
<td>22.45</td>
<td>1.035</td>
<td>0.235</td>
</tr>
<tr>
<td>Garlic extract</td>
<td>19.89</td>
<td>1.012</td>
<td>0.127</td>
</tr>
</tbody>
</table>

SD=Slandered Deviation, SE=Slandered error. ANOVA, P<0.001

**Discussion**

The objective of biomechanical preparation is to remove the pulp and dentinal debris along with smear layer which consists of bacteria. 7 Hence it is necessary to remove these microorganisms by root canal irrigants while cleaning and shaping of root canal. The irrigating
process has three objectives as advocated by the Walker: 1) Dissolution of remnant tissue, 2) antimicrobial action, and 3) lubrication of the canal.\textsuperscript{10} Intracanal irrigants should possess a good antimicrobial property to enhance the outcome of the instrumentation procedures.\textsuperscript{4}

Microbial load of root canal system can be decreased by mechanical preparation of the root canal and disinfection.\textsuperscript{8} Irrigation plays a major role in successful debridement and disinfection. The most widely used irrigant for root canal therapy is NaOCl at a concentration of 0.5 to 5.25\%. The tissue-dissolving capacity and microbicidal activity of NaOCl make it an excellent irrigating solution, but it has only a limited effect on dissolution of the smear layer. \textsuperscript{7} Due to its adverse effect alternative irrigants are tried.

Babaji et al evaluated the antimicrobial efficacy of Morinda citrifolia, Aloe vera, Azadirachta indica extract (herbal) with sodium hypochlorite (NaOCl). They concluded that Tested herbal medicine (A. indica extract, M. citrifolia, Aloe vera) showed inhibitory zone against E. faecalis. Hence, these irrigants can be used as root canal irrigating solutions.\textsuperscript{1}

Paul et al evaluated the efficacy of ethylene diamine tetraacetic acid (EDTA), EDTA along with ultrasonication, citric acid, and MTAD irrigants with sodium hypochlorite (NaOCl). They concluded that all the test irrigants including MTAD worked well in the middle and cervical third, whereas MTAD showed excellent results.\textsuperscript{7}

Singh et al assessed different irrigation activation system-F-File, CanalBrush (CB) and EndoActivator (EA) in removing smear layer from root canal. They concluded that CB remove smear layer more efficiently from the root canal than F-File and EA in coronal and apical region.\textsuperscript{11}

Renuka Singh et al evaluated the root canal debridement with three different irrigation systems, i.e., syringe. They concluded that Root canal cleanliness achieved by ultrasonic, especially in the apical third is significantly better than sonic and syringe irrigation.\textsuperscript{2}

Jain et al evaluated the efficacy of triple antibiotic solution containing tetracycline, ornidazole and ciprofloxacin as a new endodontic irrigant. They concluded that triple antibiotic irrigating solution can be used as an irrigating solution. The antibacterial action of triple antibiotic irrigating solution is comparable with chlorhexidine.\textsuperscript{5}
Prabhakar et al evaluated the antimicrobial efficacy of *Morinda citrifolia* juice (MCJ) with chlorhexidine (CHX) as endodontic irrigants and their effect on micro-hardness of root canal dentin. They concluded that nearly 0.2% of CHX showed the highest antimicrobial activity even after 28 days followed by MCJ, but none of the irrigants tested had any effect on the micro-hardness of root canal dentin.\(^\text{12}\)

Murugesan et al evaluated the effectiveness of three irrigation systems, namely, Endovac system, Max I probe, and Navitip FX, in reduction of *Enterococcus faecalis* population from the root canal. They found that all four tested irrigation delivery systems are effective in the reduction of *E. faecalis*.\(^\text{3}\)

Damre assessed the antimicrobial activity of neem leaf extract, turmeric, honey and aloe vera vs 5% sodium hypochlorite root canal irrigants against *E. Faecalis*. They observed that, honey had the highest zone of inhibition against E.faecalis followed by neem, sodium hypochlorite, haldi and aleo-vera.\(^\text{4}\)

Daga et al evaluated the antimicrobial efficacy of herbal irrigants neem, miswak, propolis with sodium hypochlorite using conventional needle irrigation and EndoVac irrigation system against *Enterococcus faecalis*. They concluded that Sodium hypochlorite proved to be a better irrigant followed by propolis, neem, and miswak. EndoVac irrigation system was more effective for elimination of *E. faecalis*.\(^\text{8}\)

Gupta-Wadhwa et al evaluated the intracanal bacterial reduction promoted by chemomechanical preparation using three different herbal extracts named *Ocimum sanctum* (*OS*), *Cinnamomum zeylanicum* (*CZ*), *Syzygium aromaticum* (*SA*) against *Enterococcus faecalis*. They concluded that Cinnamomum zeylanicum, Syzygium aromaticum and Ocimum sanctum showed intracanal bacterial reduction against *Enterococcus faecalis* but tested irrigants were less effective compared to sodium hypochlorite.\(^\text{13}\)

Chaudhari et al assessed the antimicrobial efficacy of sodium hypochlorite (NaOCl), Silver Diamine Fluoride (SDF), Chitosan Nanoparticles (CNPs) and Bioactive Glass Nanoparticles (BAGNP) as root canal irrigants against the bacterial strain of *Enterococcus Faecalis* (*E. faecalis*). They concluded that sodium Hypochlorite was the most effective root
canal irrigant followed by SDF and Bioactive Glass Nanoparticle whereas Chitosan Nanoparticles was the least efficacious compared to the rest against Enterococcus Faecalis.¹⁴

Nagaveni et al evaluated the antimicrobial efficacy of different concentrations of aloe vera against CHX. They found inhibitory zone against E. faecalisAloe vera. Hence, these can be used as root canal irrigating solutions.¹⁵

Shweta and Kumar compared the antimicrobial efficacy of three different type of irrigating solutions. They concluded that 5.25% NaOCl/hydrogen peroxide had a better antimicrobial action than 3% NaOCl/BioPure MTAD. These findings are similar to our results.

Roy et al assessed the effectiveness of garlic extract with conventional intracanal irrigants on Candida Albicans. They concluded that 2% Chlorhexidine showed greatest antifungal efficacy. 3% Sodium hypochlorite and Garlic extract exhibited antifungal efficacy with lower level.⁹

Agar dilution tests used in the present study is more dependable with other studies testing methods of antimicrobial action. ⁴

In the present study triple antibiotic solution has been formulated with a composition of 1% Ornidazole, 1% Ciprofloxacin and 1% Tetracycline in 100 ml of water following, the success rate of triple antibiotic paste.

Enterococcus faecalis is the most common organism cultured from failed root canals that undergo retreatment and also from non-healing endodontic cases. It is a facultative, gram positive anaerobic organism, which grows through the formation of a biofilm and can survive chemomechanical preparation, intracanal medication and can reinfect the obturated root canal.⁴Enterococcus faecalis (E. faecalis) is one of the most commonly detected and isolated microorganisms from teeth with pulpal necrosis.¹⁴Hence Enterococcus faecalis tested in the present study to evaluate the effectiveness of the tested irrigants action against it.

In the present study we found that, the tested irrigants triple antibiotic paste irrigant and MTAD showed antibacterial efficacy comparable to Sodium hypochlorite. Hence these irrigants can be used as efficient root canal irrigants.
The limitations of the present study are; the study was not carried on individual microorganisms since the micro-flora of the root canal is mixed in nature. The results obtained from individual micro-organisms may vary from those mixed in nature. Further studies are recommended to evaluate the physiochemical properties of the triple antibiotic, MTAD and garlic extract irrigating solution to improvise its efficacy in vivo.

**Conclusion**

The tested root canal irrigants, triple antibiotic paste and MTAD showed antibacterial efficacy comparable to Sodium hypochlorite. Hence these irrigants can be used efficiently in endodontic procedure.

Acknowledgement: Self

Conflict of interest: Nil

Source of Funding: Self

**References**


