ANTIMICROBIAL EFFICACY OF SODIUM HYPOCHLORITE, CHLORHEXIDINE, MTAD AND TRIPHALA AGAINST ENTEROCOCCUS FAECALIS - AN IN VITRO STUDY.

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Abstract

Introduction: The goal of root canal treatment is to reduce bacteria from the infected root canal system and to avoid reinfection. The antimicrobial efficiency of MTAD (Mixture of Tetracycline Isomer, an Acid and a Detergent), Chlorhexidine, Sodium hypochlorite (NaOCl), Triphala against *E. faecalis* (*Enterococcus faecalis*) was assessed in this research.

Materials and method: In this research, one hundred and fifty single-rooted extracted teeth were chosen. Samples were split randomly into five groups of thirty in each group. Teeth were immersed in 5.25percent NaOCl to eliminate surface soft tissue and debris. The teeth were divided horizontally into three sections. The middle segment was placed in a brain-heart infusion broth of E. faecalis culture. The samples were split randomly into five groups on the basis of irrigant used. Dentinal canal shavings were scrapped with a round bur on a sterile aluminum foil to test for bacterial survival. The standard deviation and mean Colony Forming units (CFU/mg) values were computed.

Results: The antibacterial property of MTAD was superior to NaOCl, Chlorhexidine and Triphala againstE. faecalis. Post hoc analysis showed a significant difference in all four experimental groups

Conclusion: MTAD had increased antibacterial action when compared to 2percent Chlorhexidine, 3percent Sodium hypochlorite, and Triphala against *E. faecalis*.

Keywords: *antibacterial, Chlorhexidine, E. faecalis, MTAD, Sodium hypochlorite, Triphala*

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INTRODUCTION:

Microorganisms have a vital role in the etiology of periapical and pulp disorders. The healing of periapical tissues is mostly determined by root canal disinfection. Clinical investigations have shown that using a chemo-mechanical preparation as an adjuvant to antimicrobial medicines is efficient in decreasing the bacterial load in the root canal[1].

Though, some microorganisms are resistant to these attempts *E. faecalis* (*Enterococcus faecalis*) is the most common kind of facultative anaerobic bacteria.*E. faecalis* is persistently observed in root canal failures and may exist in the root canal as a single species or as a significant element of the flora that is resistant to several intracanalmedicaments[2].

Nontoxicity, antimicrobial activity, and tissue dissolving capacity to the periapical region are all significant characteristics of an endodontic irrigant[3].

Antimicrobial activity and the capability to dissolve necrotic tissue make sodium hypochlorite (NaOCl) the most often utilized root canal irritant but it has an unpleasant taste and odor, corrosive potential, and a tendency to bleach clothes. It doesn't disinfect the root canal system consistently and doesn't eliminate the "smear layer" from the dentinal walls. Furthermore, when extruded into the periradicular tissues, it is very poisonous[4]. Consequently, a similarly effective but safer irrigant is needed.

Dental caries and periodontal disease may be prevented and treated with the use of CHX (Chlorhexidine) as a mouth rinse. It is used as irrigating solution or intra canal dressing in endodontic treatment[5]. CHX exhibits a property of substantivity and has a low toxicity grade but is ineffective against necrotic tissue[6]. A seek for a superior root canal irrigant is ongoing due to these constraints.

An irrigating solution, MTAD ("Mixture of Tetracycline Isomer, an Acid, and a Detergent") has revealed promising outcomes. Latest studies have revealed that it may safely eliminatethesmearlayerandremove*E.Faecalis*[7].

TheantimicrobialpropertyofMTAD was substantially more efficient than Ethylene diamine tetra acetic acid (EDTA) and NaOCl with the standard in vitro microbiologic approaches.

Researchers are looking for natural alternatives because of the ongoing rise in antibiotic-resistant bacteria and the negative impacts of synthetic medications. Triphala constitutes powdered and dried fruits of 3 medicinal plants *Emblica Officinalis, Terminaliabellerica,* and *Terminaliachebula*[8].The goal of the invitro analysis was to compare the antimicrobial effectiveness of 2 percent Chlorhexidine, MTAD,3percent NaOCl & Triphala against *E.faecalis* in this study.

METHODOLOGY:

The study was conducted after institutional ethical clearance (NO.IEC/2021-22/S-14) and it was conducted in accordance with the Declaration of Helsinki. In this in-vitro experimental analysis, freshly extracted, one hundred and fifty single-rooted teeth were chosen as samples and they were split into five groups of thirty in each.

Single rooted teeth were immersed in 5.25 percent NaOCl to eliminate organic debris and surface soft issue. A carbide disc in a straight handpiece was used to segment the teeth horizontally into apical, middle, and coronal sections. The5mm middle segment was used for this study. To standardize the internal diameter of the canal, each specimen had its root canal expanded with a # 10 round bur. The smear layer, including inorganic and organic debris, was eliminated using a 17% EDTA solution followed by 5 minutes of washing with 5.25percent NaOCl each. The segments were then autoclaved at 121°C for 30minutes to sterilize them. To infect the dentinal tubules, the segments were put in a "brain-heart infusion" broth with an E. faecalis culture and cultured at 35°C for 5 days. After that, the segments were washed in sterile water and blotted dry using sterile gauze. The root specimens were separated into 5 groups and pasted in Petridishes using a quick setting epoxy resin.

Group 1: (30 samples) 3% sodium hypochlorite was put into the segment's canal space.
Group 2: (30 samples) 2% Chlorhexidine was put into the segment's canal space.
Group 3: (30 samples) BioPure® MTAD was put into the segment's canal space.
Group 4: (30 samples) Triphala was put into the segment's canal space.
Group 5: 30 samples were kept as a control in which saline was placed.

For one week, the groups were incubated at 35° C with 100 percent humidity. The segments were removed from the Petri dishes after 1 week and irrigated with 2ml sterile water before being dried with gauze and paper points.

Dentinal shavings from inside the canal were gathered using round burs of increasing diameter, placed on a piece of sterile aluminum foil, and weighed to determine bacterial survival. In a solution, the dentinal shavings were suspended. $1/100^{th}$ of this was pipette out and poured onto McConkey'sagar. This was streaked using a sterilized metal loop to disperse the suspension uniformly around the agar plate. (figure 1,2,3,4,5)CFU ("Colony-Forming Units") were measured after they had been incubated for 24 hours. The number of CFU/mg of dentin was calculated based on the weight of recorded dentinal shavings. For each group, the mean CFU/mg as well as SD ("Standard Deviation") was computed.

Figure 1 : Colony Forming unit of Group 1(Sodium hypochloride) on McConkey's Agar

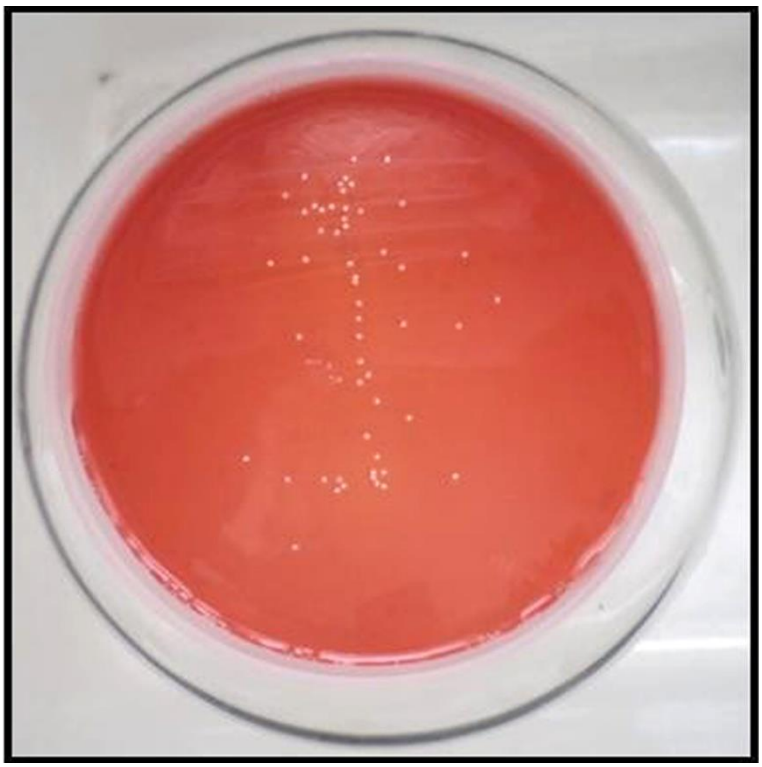

Figure 2: Colony Forming unit of Group 2(Chlorhexidine) on McConkey's Agar

Figure 3 : Colony Forming unit of Group 3 (MTAD) on McConkey's Agar

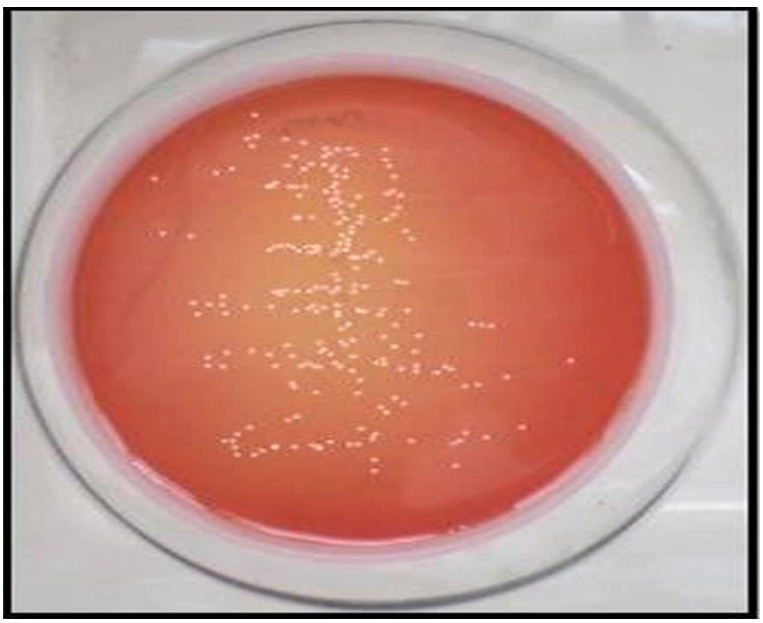

Figure 4: Colony Forming unit of Group 4 (Triphala) on McConkey's Agar

Figure 5: Colony Forming unit of Group 5(Control) on McConkey's Agar

STATISTICAL ANALYSIS:

Data were depicted in terms of mean and SD ANOVA ("Analysis of Variance"). It was used to examine antimicrobial effectiveness in all groups, followed by a post hoc test and a "Dunnett's t-test" compared with the control group. P-values below 0.05 were deemed significant. Data analysis was conducted with Minitab v14.0 software (National Institute of Standards and Technology, Pennsylvania State University, USA)

RESULTS:

Comparison of all groups shows statistically highly significant ($p<0.0001$). A post hoc study reveals that all four experimental groups show a substantial variation from the control group ($p<0.01$) (Table 1 and Table 2). Based on the result, group 3 showed better antimicrobial efficacy among all the groups.

Table:1 Basic characteristics of groups

	N	Min	Max	Mean	SD	SE
Group 1	30	56x 10^3	92x 10^3	74.8x 10^3	9.91x 10^3	1.8x 10^3
Group 2	30	72x 10^3	114x 10^3	92.8x 10^3	10.23x 10^3	1.86x 10^3
Group 3	30	36x 10^3	82x 10^3	49.3x 10^3	11.65x 10^3	2.13x 10^3
Group 4	30	118x 10^3	168x 10^3	145.1x 10^3	12.56x 10^3	2.29x 10^3
Group 5	30	162x 10^3	182x 10^3	172.2x 10^3	7.63x 10^3	2.41x 10^3

(N-Sample size, Min-minimum, Max-maximum, SD-standard deviation, Standard Error)

Table:2 Comparison of antimicrobial efficiency of MTAD, CHX, NaOCl Triphala against Saline in dentinal tubules of human teeth (mean±SD)

	"Group 1	Group 2	Group 3	Group 4	Group 5	F value	p-value"
CFU/mg (103)	74.8 ± 9.9 **	92.8 ± 10.2 **	49.3 ± 11.6 **	145.1± 12.6**	172.2 ± 7.6	439.1	P<0.0001

(CFU- colony forming units)

* $p<0.05$, **$p<0.01$, compared to control, Dunnett's t-test is employed after ANOVA.

DISCUSSION:

Human teeth have complicated root canal morphology with multiple fins, cul-de-sacs, as well as lateral canals. Bacteria can be found at different depths in the irregularities and dentinal tubules[9].

Irrigation ensures the removal of bacteria and the eradication of organic tissue debris. NaOCl is the most popular "root canal irrigant" currently in use. It gives an effective tissue solvent and has a wide variety of antimicrobial activity, serves as a lubricant for equipment and may remove loose debris through the rootcanals[10].NaOCl was utilized as a root canal irrigant in various concentrations ranging between 0.5 percent to 5.25 percent in endodontics for a long time. Its antimicrobial action increases as the drug concentration increases. As the concentration of NaOCl increases, the antimicrobial and the cytotoxic effect of the solution on the periapical and periodontal tissues also increases. Though it is an efficient antibacterial agent, NaOCl has an unpleasant taste, odor and is harmful when injected into the periradicular tissues[11].So an alternative, Chlorhexidine gluconate has been used in this study.

Chlorhexidine is a cationic bis-biguanide with wide spectrum antibiotic activity and has been widely used in dentistry. CHX easily dissociates at physiological, pH releasing positively charged molecules that bind to negatively charged groups of phosphate in the microbial cell walls of the bacteria, and alters the cell's osmotic equilibrium. CHX has a unique ability to absorb into the dentin and prevent microbial colonization (substantivity) overtime[12]. In this research 2 percent CHX was found to be less efficient than 3%NaOCl.This conclusion is similar to that of Abdullah et. al, who developed "*E. faecalis"* biofilms using membrane filters[13].These findings contrast with those of Oncag et al, who observed that 2percent CHX was more efficient than 5.25 percent NaOCl at 5 min and 48 hr[14].These results are consistent with Gomes et al and Vianna et al where 0.2%, 1%, 2% CHX gel, and liquid eliminated *E. faecalis* in 1 min[11,15]. The antimicrobial action of irrigants is dependent on their kind, presentation, as well as concentration form, and microorganism susceptibility. The action of CHX is pH-related and drastically decreased in the existence of organic materials. One of its greatest

disadvantages is that it lacks the tissue dissolving characteristics of NaOCl.

The healing ability of plants is an old phenomenon, but recently it has obtained renewed attention and significance.

Triphala is an ancient Indian natural formulation comprising powdered and dried fruits of 3 medicinal plants *E. officinalis, Terminaliabelleric* and *Terminaliachebula.* Tannic acid is the main component of the ripe fruit of *E. officinalis, T. belerica*, and *T. chebula*. Few investigations have shown that tannic acid is bactericidal or bacteriostatic to some gram-positive as well as negative pathogens. Triphalais known to be safe, with active constituents showing anti-inflammatory, antioxidant and radical neutralizing activity[16,17]. Moreover Triphala is a known chelator. It includes fruits high in citric acid, which could help with smear layer removal. *T. chebula*one of the constituents of Triphala has been revealed to have a broad range of biological activity, like antifungal, anticancer, antibacterial, antiviral activities, *etc*. Its methanol and acetone extracts have shown antioxidant activity. Natural herbal alternatives are easily available, inexpensive with lowtoxicity[17].

In a study conducted by Prabhakar et al to determine the antimicrobial efficiency of herbal alternatives (Green tea polyphenols and Triphala), MTAD and 5 percent NaOCl against *E. faecalis* biofilm on tooth substrate, Sodium hypochlorite indicated the highest antibacterial against 3 & 6-week biofilm. MTAD and Triphala indicated biofilm eradication in 3-weeks[18].In the current research Triphala was revealed to have an antimicrobial effect against *E.faecalis*.

MTAD, after its introduction in 2003 was evaluated for its efficiency and compared to a variety of frequently used irrigantion solutions. The enhanced MTAD antimicrobial activity over 3percent NaOCl found in the present study areconsistent with the results of Torabinejad and Shabahang's investigation[19,20].

E. faecalis was selected in the current research because it is capable of penetrating as far as 250μm into the dentinal tubules thus enabling it to resist the action of irrigants. *E. faecalis*, a Gram-positive facultative anaerobic micro organism was chosen in this study as it is frequently observed in the failed root canal treated cases and is resistant to presently utilized chemicals like Calcium hydroxide, Potassium iodide, or NaOCl and was observed to sustain asa mono-infection in the root canals[2,4,10].

To enable bacteria to enter the tubules, the smear layer has been eliminated before the teeth were contaminated with *E. faecalis* in the current investigation. Vitro examinations have demonstrated a high degree of *E. faecalis* susceptibility to MTAD, even in 200 dilutions, but NaOCl loses its antibacterial action after 32 times dilution against the same isolate[5]. MTAD eliminates the smear layer better as compared to EDTA.[7] MTAD was found to be less cytotoxic than EDTA, 5.25 percent NaOCl, and 0.12 percent Chlorhexidine gluconate[21].

The antimicrobial efficiency of MTAD is due to Doxycycline's anti-collagenase action, removing debris with citric acid, and its capacity to be delivered gradually over time[7]. Tween-80 the detergent present in MTAD declines the surface tension of the dentinal tubules facilitating Doxycycline penetration into the tubules. MTAD has the ability to kill *E. faecalis* after just 5min of exposure making it highly advantageous in the clinical situation. Newberry etal. concluded that MTAD reduced the *E. faecalis* growth[19].Thus MTAD has superior antibacterial activity.

The finding of the current research corroborated the results of Davis et al, who showed 1%, 6percent NaOCl was more efficient in decreasing *E. faecalis* biofilms than Biopure® MTAD[22].Differences in technique and variation in strains examined might explain the inconsistency in the results of the above studies.

In the current research, *E. faecalis* grown was in planktonic form while Dunavant etal. used biofilms[23].Torabinajad's group suggested using 1.3 percent NaOCl for15-20minutes before the final rinse using MTAD[7]. Some research did not utilize NaOCl at all or only utilized it for a lesser time than suggested. The variation between the biofilm cultures and planktonic culture is that all bacteria cells inside the biofilm are not equal. On the basis of the findings of our present research, it may be found that the MTAD has superior bactericidal activity against *E.faecalis* compared to 2 percent CHX, 3 percent NaOCl and Triphala. This is in line with the investigations done by

Torabinejad etal[24]. Further in vivo & in vitro investigations are necessary to find out the efficacy of MTAD against various other organisms observed in the root canal system because root canal infections are mixed infections.

CONCLUSION:

The antibacterial activity of the tested irrigant depends on the concentration, form as well as microbial susceptibility. The true determinant of the efficacy of the irrigant is its effectiveness in the clinical situation, so further in vivo studies with longer follow-up should be performed to assess the efficiency of MTAD. Within the constraint of this present in vitro analysis, the following conclusions were drawn:

1. BioPure® MTAD inhibits the growth of *Enterococcus faecalis* when used as an intracanal irrigant.
2. Its antibacterial activity is superior to that of 3%NaOCl, 2%Chlorhexidine and Triphala.
3. 3% NaOCl was more efficient than 2% Chlorhexidine and Triphala against *E. faecalis*.

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