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Original Article

Dentistry Section

Antimicrobial Efficacy of Tubulicid Red as a Cavity Disinfectant in Comparison with Chlor-X Gel against *S. mutans* in Primary Teeth: Randomised Split-mouth Clinical Study

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ABSTRACT

Introduction: Microorganisms act as precursors to dental caries. Following cavity preparation, traces of resident bacteria in the smear layer may contribute to the recurrence of caries. Thus, eliminating the underlying causes improves the likelihood of treatment success. Disinfectants are adjuvant tools that should focus on removing microorganisms when combined with procedures that yield positive outcomes.

Aim: To compare and evaluate the antimicrobial efficacy of Tubulicid Red (TR) Label as a cavity disinfectant with Chlor X gel (2% chlorhexidine) in primary teeth.

Materials and Methods: The present randomised double-blind clinical trial was conducted at the Department of Paediatric and Preventive Dentistry, GSL Dental College and Hospital, YSRUHS, Rajahmundry, Andhra Pradesh, India from September 2021 to April 2022. The study assessed the antimicrobial efficacy of TR label against *S. mutans* in 60 primary carious teeth involving 1/3rd of the dentin. The teeth were divided into two groups, each consisting of 30 teeth. Group-I received TR Label, while Group-II received Chlor X. After excavating the infected dentine, the affected dentine was left in the cavity, and a dentinal sample was collected from the base of the cavity. The cavity was then disinfected with either TR or Chlor X gel, followed by rinsing with saline. A second sample was collected from the base of the same cavity. The collected samples were transferred to Eppendorf tubes and subjected to microbiological analysis to calculate the number of Colony-forming Units (CFU) of *S. mutans* on Mutans Sanguis (MS) agar medium. The obtained data were subjected to statistical analysis using the Wilcoxon's signed-rank test and Mann-Whitney test.

Results: In the intragroup comparison (Wilcoxon's signed-rank test), both groups showed significant reductions in *S. mutans* count ($p<0.001$). The intergroup comparison showed no statistically significant difference ($p>0.05$) between the Tubulicid and Chlor X groups at baseline and post-treatment.

Conclusion: The TR could be used as an alternative to Chlor X gel as a cavity disinfectant in primary teeth before restoration, potentially reducing the occurrence of secondary caries.

Keywords: Microorganisms, Mutans sanguis agar medium, Secondary caries, *Streptococcus mutans*

INTRODUCTION

Dental caries is a bacterial infection that causes the localised breakdown of dental hard tissues through acidic byproducts produced during the bacterial fermentation of dietary carbohydrates [1]. Initially, Grieve AR claimed that the bacteria that play a major role in the development of caries also play a substantial role in the establishment of secondary caries [2]. Later, Kidd EA and Beighton D confirmed this by taking samples from the dentinoenamel junction, which showed higher levels of *S. mutans* streptococci and lactobacilli under restorations. No significant difference was observed between samples derived from secondary caries lesions and those from primary lesions [3].

However, mechanical preparation of the tissue is not sufficient to completely eliminate the bacteria that may remain in the smear layer, on cavity walls, at the enamel-dentine junction, and in the dentinal tubules. Literature (Brannstrom M [4] & Demarco) has revealed that the microorganisms left in the smear layer can multiply even in the presence of a tight seal in the oral cavity. This can lead to the development of secondary caries, postoperative sensitivity, pulpal damage, and discolouration due to microleakage, ultimately resulting in treatment failure. Thus, eliminating the root cause enhances the success rate of the treatment.

Disinfectants are one of the adjunct tools used to inhibit the growth of residual bacteria in deep cavities. Several researchers have evaluated the use of antimicrobial agents such as iodine povidone, hydrogen peroxide, sodium hypochlorite, and Chlorhexidine Gluconate (CHX) as cavity disinfectants before restoration. Chlorhexidine is a well known antibacterial chemical employed as a cavity disinfectant, and studies have shown its effectiveness in reducing residual bacteria in dental tissues [5,6].

Recently, a disinfectant called TR, composed of "Benzalkonium Chloride (BAC)," has exhibited antibacterial effects without compromising the bonding capacity, which has shed light on restorative treatment procedures [7]. However, the efficacy of this disinfectant still needs to be evaluated in a wide range of scenarios with pre- and post-application outcomes. Based on the reviewed literature, present study is the first in-vivo study to compare the antimicrobial efficacy of TR with Chlor X gel in primary teeth.

MATERIALS AND METHODS

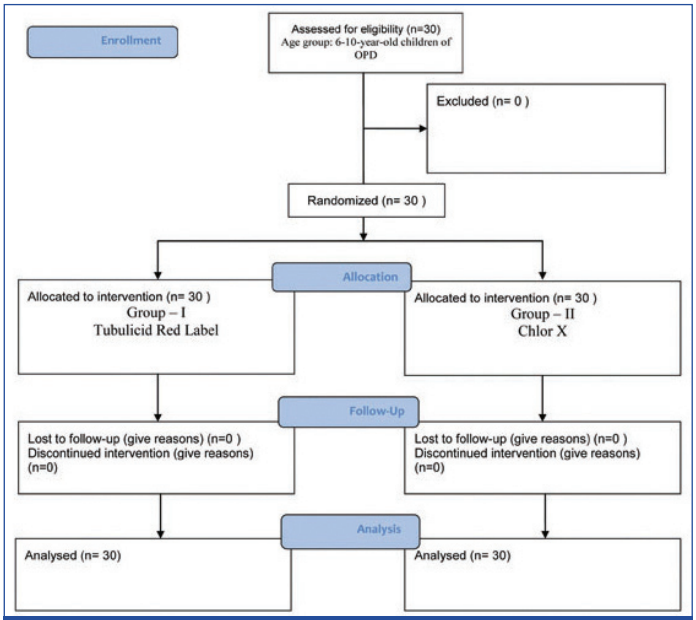
The present study was a randomised, double-blind clinical trial conducted on 30 children, who reported with occlusal caries bilaterally in primary 1st and 2nd deciduous molars to the Department of Paediatric and Preventive Dentistry, GSL Dental College and Hospital, YSRUHS, Rajahmundry, Andhra Pradesh, India between September 2021 and April 2022. The study was approved by the Institutional Ethical Committee (IEC Ref No: GSLDC/IEC/2021/011). The trial was registered at the Clinical Trials Registry-India (CTRI/2021/09/036856). The sample size was calculated with GPower software and it was set at a power of 80. This gave us a value of 30 sample size. A letter of informed consent, providing all the information about the study, was given to the parent/guardian, and only children with signed written consent were included.

Inclusion criteria: The subjects who had bilateral caries in primary 1st and 2nd molar teeth involving 1/3rd of dentine and radiographic examination revealed caries affecting the occlusal surface (Class-I) of primary molars with more than 2 mm of residual dentine thickness were included in the study.

Exclusion criteria: Patients who had non restorable teeth, perforated pulpal floor, excessive mobility, or pathological root resorption or caries involving the pulp were excluded from the study.

Study Procedure

Dentinal sample collection and inoculation: After clinical and radiographic confirmation, the subjects were allocated to the test or control group using a simple lottery method. The subjects who picked even numbers first were allocated to Group-1 (TR label group), while those who picked odd numbers first were allocated to Group-2 (Chlor X gel group) in the first visit [Table/Fig-1].

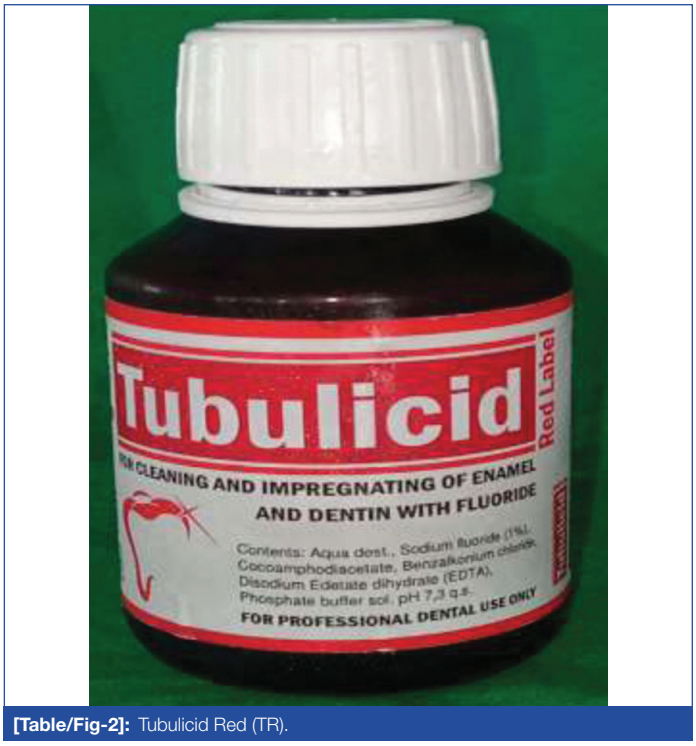


[Table/Fig-1]: Consolidated Standards of Reporting Trails (CONSORT) flowchart.

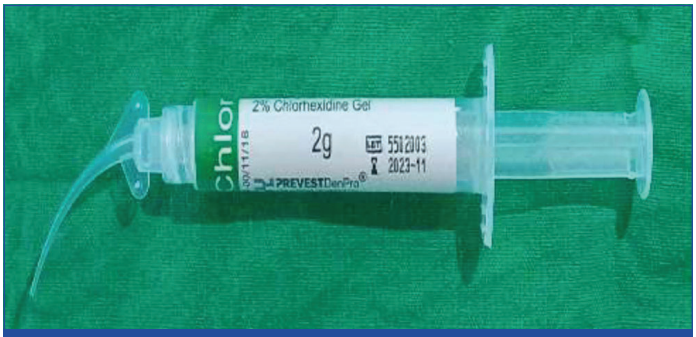
The tooth isolation (maxillary/mandibular primary molars) was done with a rubber dam. The carious lesion was then excavated using either a slow-speed contra-angle handpiece (Appledental) or a sterile sharp spoon excavator (#3000, API India). After removing the infected dentine, which was light brown in colour, soft, and leathery in consistency, the hard, dark brown affected dentine was left behind. The dentinal sample (baseline/S1) was collected by scraping the base of the cavity using a sterile sharp spoon excavator. After caries excavation, the subject was allocated to one of the groups using a simple lottery method.

Approximately 1 mL of cavity disinfectant, either TR label (0.1% Blood Alcohol Content (BAC) or Chlor X gel (2% chlorhexidine) [Table/Fig-2,3], was applied to the cavity using an applicator tip for 60 seconds. After disinfection, the cavity was rinsed with sterile distilled water, and a second sample (S2) was taken from the base of the cavity. The collected sample was stored in Eppendorf tubes containing 0.5 mL of phosphate buffer solution and subjected to microbial evaluation [8]. All the procedures, including excavation, sample collection, and final restoration, were performed by a trained paediatric dentist who was blinded to the groups. The subjects who participated in the study were also blinded.

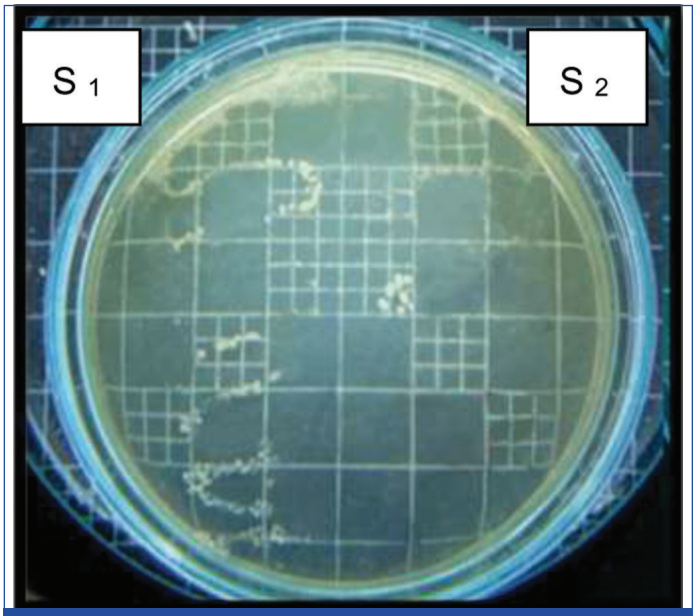
Microbial evaluation: A 2 µL dentinal samples S1 and S2 were inoculated on two halves of the MS agar plate, which is a selective medium for *Streptococcus mutans*. The plates were incubated for 72 hours in an atmosphere containing 5% CO_2 and 95% N_2 at 37°C. Colonies that appeared grayish-yellow in colour on the incubated plates were counted using a digital colony counter (CFU/mL) [Table/Fig-4,5] [8].



[Table/Fig-2]: Tubulicid Red (TR).



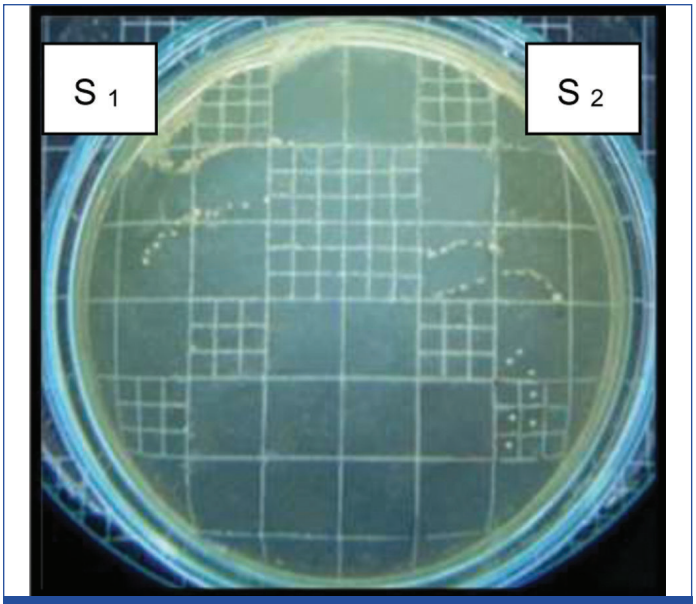
[Table/Fig-3]: Chlor X gel (2% chlorhexidine).



[Table/Fig-4]: Pre (S_1) and post (S_2) growth of *S.mutans* after application of Chlorhexidine Gluconate (CHX) on Mutans Sanguis (MS) agar media.

STATISTICAL ANALYSIS

In present study, the statistical analysis was performed using Statistical Packages for Social Sciences (SPSS) software. Intragroup analysis was conducted using the Wilcoxon's signed-rank test. The Mann-Whitney U test was utilised to compare the difference between the means of the two groups. A p-value of less than 0.05 ($p<0.05$) was considered statistically significant.



[Table/Fig-5]: Pre (S_1) and post (S_2) growth of *S.mutans* after application of TR on Mutans Sanguis (MS) agar media.

RESULTS

The study included children between the ages of 6 and 10 years. In the TR group, the mean colony count at baseline was 82,000 CFU/mL×10^3, and after treatment, it was 14,400 CFU/mL×10^3 [Table/Fig-6]. In the Chlor X group, the mean colony count was 86,966.7 CFU/mL×10^3 at baseline and 14,266.7 CFU/mL×10^3 after treatment [Table/Fig-7]. There were significant reductions in the *S. mutans* count after cavity disinfection in both groups (p<0.001).

Variables	N	Baseline mean (CFU/mL)×10^3	Final mean (CFU/mL)×10^3	p-value
Tubulicid Red (TR) label	30	82.000	14.4000	<0.001*

[Table/Fig-6]: Comparison of baseline and final mean CFU/mL of TR group.
*Statistically significant, Wilcoxon's signed-rank test

Variables	N	Baseline mean (CFU/mL)×10^3	Final mean (CFU/mL)×10^3	p-value
Chlor X group	30	86.9667	14.2667	<0.001*

[Table/Fig-7]: Comparison of baseline and final mean CFU/ml of Chlor X group.
*Statistically significant, Wilcoxon's signed-rank test

The intergroup comparison using the Mann-Whitney U test showed no statistical difference in *S. mutans* count at baseline (p-value=0.723) and post-treatment (p-value=0.641) [Table/Fig-8]. Based on the results, tubulicid can be considered as an alternative to chlorhexidine for cavity disinfection in primary teeth.

Time interval	Group	Mean (CFU/mL)×10^3	Median (CFU/mL)×10^3	Standard Deviation (SD) (CFU/mL)×10^3	Z-value	p-value
Baseline	Tubulicid Red (TR) label	82.000	73.0000	46.00675	426.000	0.723
	Chlor X group	86.9667	76.5000	49.47621		
Final	Tubulicid Red (TR) label	14.4000	12.5000	10.65315	418.500	0.641
	Chlor X group	14.2667	10.0000	12.21343		

[Table/Fig-8]: Intergroup comparison of bacterial count (CFU/mL).
Mann-Whitney U Test

DISCUSSION

Tooth decay is a microbiological disease that develops due to an imbalance between tooth minerals and plaque [9]. Before restoring a decayed tooth, the primary goal of caries removal is to eliminate the soft, infected, and demineralised dentinal tissue while protecting the healthy and remineralised tissue at the base of the cavity [10]. According to Brannstrom M [4], bacterial microleakage, which refers to the presence of bacteria in the smear layer, dentinal tubules, and at the dentinoenamel junction, as well as bacterial recontamination of a prepared cavity before placing a restoration, can be potential sources of infection in the prepared cavity [4]. Therefore, it is crucial to eliminate residual microorganisms present after caries removal. Conventional caries removal methods may not completely eliminate all bacteria from the cavity. Hence, the use of a cavity disinfectant after cavity preparation to reduce or eliminate bacteria has become common practice. Brannstrom M and Nyborg H were the first to propose the concept of teeth disinfection and recommended a BAC-based disinfectant for cavity disinfection [11].

In present study, TR, a BAC-based material, and Chlor X gel, which contains 2% Chlorhexidine (CHX), were tested against this cariogenic bacterium. Both materials demonstrated equal efficacy in reducing colonies of *S. mutans*.

The 2% CHX group was considered the control as it has been established as the gold standard for cavity disinfectants [12]. Since CHX solution can be cytotoxic at high concentrations, gel forms are recommended as they have shown less cytotoxicity, greater biocompatibility, and long-term antibacterial effects in deep dentine cavities [13]. This is supported by a study conducted by Ferraz CC et al., comparing the antimicrobial efficacy of chlorhexidine gel, chlorhexidine solution, and sodium hypochlorite, which concluded that chlorhexidine gel exhibited greater antimicrobial potential than the other agents used in the study [14].

In a study by Pattanaik N and Chandak M, TR showed a greater percentage reduction in *S. mutans* count in dentinal samples taken before and after the application of a cavity disinfectant [15]. These results are consistent with the findings of the present study. TR primarily consists of 0.1% BAC, 0.2% Ethylene Diamine Tetraacetic Acid (EDTA), and 1% sodium fluoride. BAC is a nitrogenous cationic surfactant that contains a quaternary ammonium group and exhibits good wettability [16]. It acts as an antimicrobial agent (bactericidal against gram-positive and some gram negative bacteria) and a matrix metalloprotease inhibitor [17].

Several studies [10,15,18] have demonstrated the bactericidal effects of Tubulicid against *S. mutans*. Mejàre B et al., compared Tubulicid-treated cavities with cavities that were not treated with a cavity cleanser and found microorganisms only in samples from untreated cavities before restoration placement [19]. Similar results were observed in present study, where teeth treated with TR label exhibited antimicrobial properties.

In an in-vitro study conducted by Bakır S et al., the antibacterial activities of different cavity disinfectants were compared, and it was observed that a cavity cleanser containing CHX and TR Label solutions containing BAC showed higher antibacterial activity against *S. mutans* [10]. Similar results were found in present study, where the cavities were disinfected with TR and Chlor X. In the present study, the count of *S. mutans* was evaluated using selective media. MS agar, a novel medium for the growth of *S. mutans* used in present study, has also been utilised in experiments conducted by Uday P et al., and Bonecker M et al., [8,20].

The results of the present study demonstrated a statistically significant reduction in bacterial count with the application of TR and Chlor X. However, it is worth noting that Chlorhexidine-based disinfectants are known to cause taste alteration, contact dermatitis, and desquamative gingivitis, which were not observed with TR [18].

Limitation(s)

In the present study, only the count of *S. mutans* was measured. Other microorganisms, such as *Lactobacillus*, which are present in the deep dentinal cavities, can also be measured. A larger sample size can yield significant results.

CONCLUSION(S)

The present study demonstrated no significant difference between the two disinfectants. Thus, it can be concluded that TR can be used as an effective alternative to Chlor X for cavity disinfection.

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