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# Antibacterial activity of Conventional and Modified Glass Ionomer Cement against *Streptococcus mutans*

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**ABSTRACT**

This study evaluates the antibacterial effects and increase in the antibiotic spectrum properties of conventional glass-ionomer cements (GIC) and the modified GICs with three antimicrobial chemical compounds. Three chemical compounds such as Boric acid, Chloroxylenol and Thymol were added to the GIC powder in the concentration of 2 % and 5 % for its antagonistic action against *Streptococcus mutans* (MTCC - 497). The antibacterial activity of the modified GICs with three chemicals was evaluated six days for its inhibitory activity against *Streptococcus mutans* by Kirby-Bauer agar well diffusion method. All the modified GICs samples have showed the increased level of inhibition compared to the ionomer cement without the chemical compounds. The compound Thymol has showed maximum inhibition, followed by Chloroxylenol and then Boric acid. Zone of inhibition was greater at 5% concentration for Thymol and Chloroxylenol. Where, Boric acid has shown maximum inhibition at 2% concentration. Thus the use of antimicrobial chemical compounds along with glass ionomer cement has provided higher antibacterial effect against the *Streptococcus mutans* and therefore can be used for as an alternative for the treatment of dental caries.

## 1. INTRODUCTION

Microbial infection is the major reason for the inflammation of the dental pulp and periodontium [1]. Recent studies indicate that the existence of residual traces of infection in the site affects the success of restoration [2]. Several species of bacteria has been isolated from dental plaque, such as *Lactobacilli, Streptococcus mutans*, *Streptococcus sobrinus* etc [3], which may induce the formation of caries. *Streptococcus mutans* is one of the most frequent bacteria involved in dental caries [4]. These cariogenic bacteria could degrade fermentable carbohydrates to acids to demineralise tooth tissue [5].

Amongst the dental restorative materials used in dentistry, the conventional glass ionomer cement (GIC) has been found to have antibacterial effects. Glass ionomer and composite based orthodontic materials are the most commonly used materials for bonding teeth with orthodontic bands and matrices. It was reported that the population of *Streptococcus mutans* (*S. mutans*) on the surface of GIC fillings was lower than on composite resin fillings [6, 7]. Glass ionomer cement has a continuous release of fluoride ions, which act as anti-cariogenic agent and helps in prevention of various oral problems like enamel demineralisation, remineralisation or by interfering with the growth or metabolism of remaining cariogenic bacteria [8, 9, 10]. Using Glass ionomer based orthodontic banding agents can reduce this problem to a certain extent. It is effective against a few, but not all oral pathogens causing dental cariogenic and periodontal problems. The spectrum of bacteria inhibited by fluoride being limited, various modifications of Glass ionomer cements have been suggested by different studies to enhance its antimicrobial properties which would help to reduce caries, plaque accumulation and periodontal problems.

Chloroxylenol (4-chloro-3,5-dimethylphenol; *p*-chloro-*m*-xylenol) is chemical compound used in antiseptic or disinfectant action [11]. Chloroxylenol is bactericidal [11]. Surprisingly, its mechanism of action has been little studied despite its widespread use over many years. Because of its phenolic nature, it has been expected to have an antimicrobial effect [12].

Boric acid or orthoboric acid ($H_3BO_3$) is a weak acid of boron. Boron is a chemical compounds which is an essential micronutrients for many organisms [13]. However, in large amounts, boron is also toxic to living cells. The gap between boron deficiency and toxicity is fairly small for all living organisms [14, 15]. Boron is involved in quorum sensing, an important mechanism in establishing antimicrobial activity [16, 17]. Thymol, phenolic monoterpenes, isolated from Thymus vulgaris, [18, 19, 20, 21] have been shown to possess antimicrobial and antioxidant activities [22, 23, 24, 25, 25, 26]. The monoterpene Thymol has been found effective against both positive and negative bacteria [27].

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The purpose of this study to enhance the antimicrobial properties of conventional Glass ionomer cements by adding with three antimicrobial chemical compounds.

## MATERIALS AND METHOD

### *Streptococcus mutans*

*Streptococcus mutans* was obtained from MTCC (497) Chandigarh, subcultured in Trypticase Soya broth (TSB) and maintained on Trypticase Soya agar (TSA) slants at 37◦C. Purity of the culture was checked by Gram's staining.

### Glass ionomer cement

The conventional restorative GIC powder and the cement liquid (Fuji IX, GC, Tokyo, Japan) was used. GIC obtained was slightly modified by mixing of 2 % & 5% of three chemical compounds (Thymol, Chloroxylenol and Boric acid) to GIC powder which is used as cement in filling of cavities of the tooth during root canal treatment.

### Preparation of inoculum

A sterile loop full of the pure culture of *Streptococcus mutans* (MTCC 497) was inoculated in to the 10ml of Trypticase Soya Broth and incubated at 37ºC for 24 hours. Growth was observed and purity was checked by Gram staining.

### Blood agar plates

Sterile ready to use Blood agar plates were obtained from Himedia, Bangalore and used for this sensitivity test. Plates were pre incubated in a 37°C incubator to check for contaminations. The plates were appropriately labeled for each chemical to be tested.

### Antimicrobial activity

Using a sterile swab, the 24 hours well grown *Streptococcus mutans* was spread uniformly on the surface of a Blood agar plates to produce a lawn. Four wells of about 0.6mm diameter were made in all plates with a sterile cork borer. The viscosity of GIC cement liquid was reduced by diluting 2 ml of cement liquid with 2 ml of methanol in 1:1 ratio. The cement powder was mixed with the diluted cement liquid in ratio of 1: 4 of diluted cement liquid in a test tube. 50µl of this mixture was loaded into the wells of blood agar plates. Similar dilutions were made for modified G.I.C. powders of Boric acid, Choloroxylenol, and Thymol and loaded into the wells. Results were observed for 144 hours at an interval of 24 hours and the diameter of clear zone of inhibition was measured.

## RESULTS

The present paper reports the enhanced antibacterial activity of the modified Glass ionomer cement with three chemical compounds such as Boric acid, Chloroxylenol and Thymol, against gram-positive *Streptococcus mutans* strain.

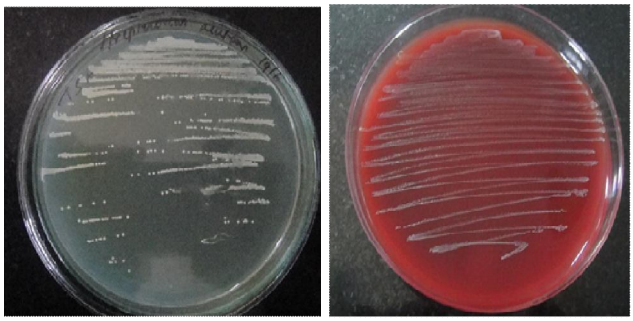

**Fig. 1:** *Streptococcus mutans* MTCC-497 on Trypticase soya agar and Blood agar plates.

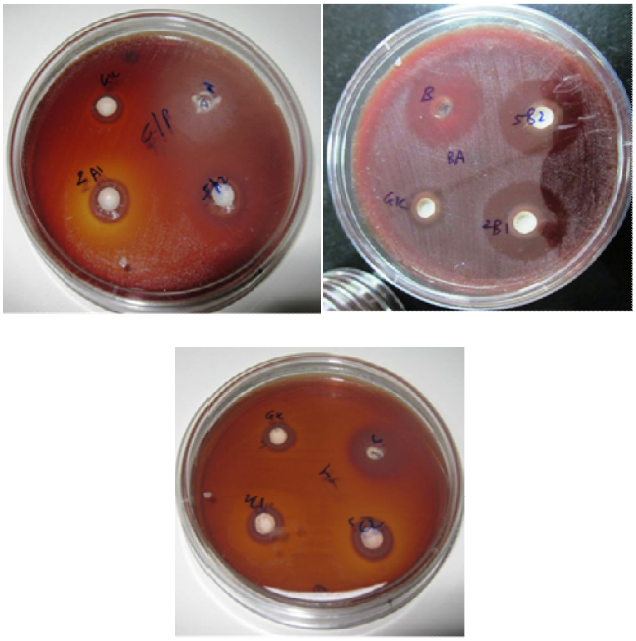

**Fig. 2:** Zone of inhibition against *Streptococcus mutans* with GIC, 2% & 5% Chloroxylenol, Boric acid, Thymol.

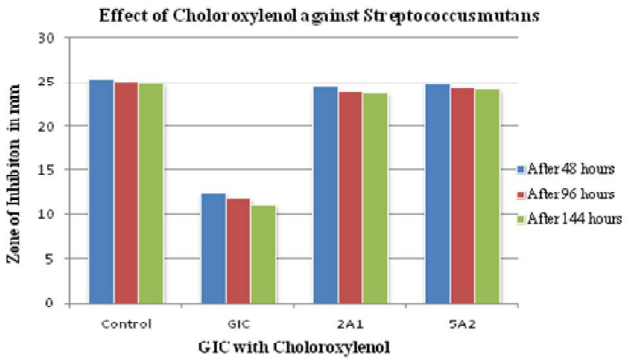


**Fig. 3:** Zone of Inhibition of Cloloroxylenol.

Control: Chloroxylenol dissolved in solvent methanol, GIC: Cement ( powder plus liquid) in methanol

2A1: 2% Chloroxylenol with cement (powder plus liquid) in methanol, 5A2: 5% Chloroxylenol with cement (powder plus liquid ) in methanol. Chloroxylenol mixture showed the maximum inhibition of 24.76 mm in diameter for 5% concentration after 48 hours of incubation.

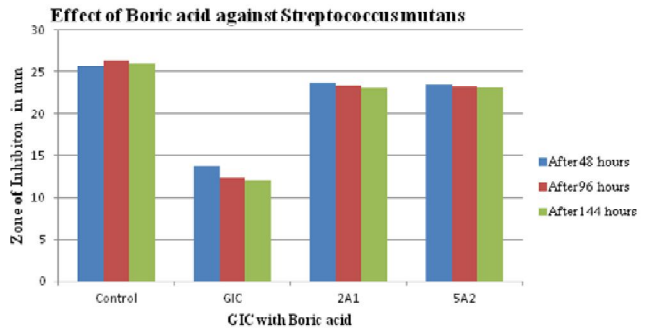


**Fig. 4:** Zone of Inhibition of Boric acid.

Control: Boric acid dissolved in solvent methanol, GIC: Cement ( powder plus liquid) in methanol 2A1: 2% Boric acid with cement (powder plus liquid) in methanol, 5A2: 5% Boric acid with cement (powder plus liquid) in methanol. Boric acid showed the maximum inhibition zone of 23.8 mm in diameter for 2% concentration after 48 hours of incubation.

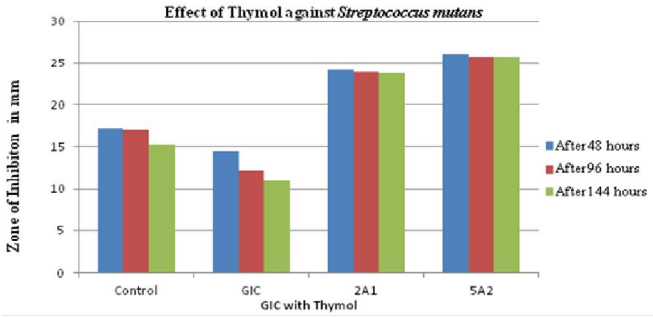


**Fig. 5:** Zone of Inhibition of Thymol.

Control: Thymol dissolved in solvent methanol, GIC: Cement ( powder plus liquid) in methanol
2A1: 2% Thymol with cement (powder plus liquid) in methanol, 5A2: 5% Thymol with cement (powder plus liquid ) in methanol. Thymol mixture exhibited maximum inhibition of 26.1 mm in diameter for 5% concentration after 48 hours of incubation.

## DISCUSSION

According to the results of the present study, pure Glass ionomer cement did not produce greater bacterial inhibition, whereas the modified Glass ionomer which mixed with Chloroxylenol, Boric acid and Thymol powder has confirmed better antibacterial activity. Thymol has showed the greater inhibition of 26.1 mm followed by chloroxylenol with 24.76 mm and Boric acid with 23.8mm in diameter. This confirms the earlier studies that the inhibition of GIC against caries formation may be because of fluoride release and/or acidity [28, 29, 30] and the inhibition of bacteria by conventional GICs in cavities is not reliable [31, 32].

The antimicrobial activity was reliant upon the concentration of the disinfectant added to GICs, [33, 34]. However, in this study, it was observed that, increasing the concentration of the antibacterial chemical compound had shown increasing effects on the antibacterial properties of the mixture against *Streptococcus mutans* for the sample Chloroxylenol and Thymol. But for Boric acid the increase in concentration has decreased the antibacterial properties. This could be because of the less or decreased reaction between the glass particles with the liquid with increased Boric acid concentration, thereby increased number of unreacted glass particles in the structure. And also the bactericidal activity of conventional and modified GIC has seems to decreasing with the number of days. The zones of inhibition in mm were found to be highest after 48 hours than 96 hours and 144 hours. This could possibly because of two reasons: One, most dental materials seem to be bactericidal while setting because in the setting process the materials had significantly more antibacterial effect due to their low pH [35]. The initial pH value after immediately mixing is considerably acidic, below 1 at which most of bacterial growth would be suppressed. Then the pH-value starts to increase to a neutral level to about 7.5 which could not inhibit the bacterial growth [36]. Second, fluoride release; the release of fluoride ions increased mostly in the 1$^{st}$ day and then decreased regularly [37]. According to Summit B, 2001 no glass ionomer cement was able to retain its acidity and fluoride ion more than 48 hours.

## CONCLUSION

The results of this in-vitro study demonstrate that modified GIC with Chloroxylenol, Boric acid and Thymol are more effective against *Streptococcus mutans* than of unmodified GIC powder. Thymol has showed maximum inhibition than Chloroxylenol and Boric acid. So, we conclude that Thymol added GIC can be a better replacement for filling of the dental cavities. Further investigations has to be carried out for the side effects and bonding effects of Thymol with GICs.

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