



ISSN Print: 2394-7489  
ISSN Online: 2394-7497  
IJADS 2021; 7(4): 82-88  
© 2021 IJADS  
[www.oraljournal.com](http://www.oraljournal.com)  
Received: 09-08-2021  
Accepted: 12-09-2021

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## Comparative evaluation of antimicrobial efficacy of bioactive glass, 1% chlorhexidine gluconate with calcium hydroxide and 1% chlorhexidine gluconate, as intracanal medicament in primary molars: An in vivo study

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DOI: <https://doi.org/10.22271/oral.2021.v7.i4b.1355>

### Abstract

**Aims:** To compare the antimicrobial efficacy of Bioactive glass, combination of 1% Chlorhexidine gluconate gel and Calcium hydroxide powder and 1% Chlorhexidine gluconate gel as intracanal medicament in primary molars.

**Settings and Design:** *In vivo* study design

**Methods and Material:** The present study was conducted in the Department of Pediatric and Preventive Dentistry, Krishnadevaraya College of Dental Sciences and Hospital, Bengaluru. This study included 48 subjects within the age group ranging from 4-11 years who were indicated for multi visit Pulpectomy. These subjects were randomly divided into three experimental groups, containing sixteen samples per group. Group 1: Bioactive glass; Group 2: 1% Chlorhexidine gel and Calcium hydroxide powder; Group 3: 1% Chlorhexidine gel. Under rubber dam isolation, following lesion sterilization S0 sample was obtained. Following access opening and working length, sample S1 was obtained and the respective medicament was placed inside the canals after cleaning and shaping and a double seal was done with ZOE and GIC. Patient was recalled after a period of 5 to 7 days and the medicaments were flushed under isolation and sample S2 was obtained. Pulpectomy procedure was then completed. S0, S1, S2 samples were subjected to microbiological analysis and the total bacterial count in CFU/ml was calculated.

**Statistical analysis used:** Kruskal Wallis Test and One-way ANOVA.

**Results:** The mean percentage of reduction of bacterial count from S1- S2 (Before and after placement of medicament) with Group 1: Bioactive glass was 75.7%, with Group 2: 1% Chlorhexidine gluconate and calcium hydroxide was 52% and Group 3: 1% Chlorhexidine gluconate was 28.6%, revealing that bioactive glass has the superior antibacterial efficacy when compared with other groups. Antibacterial efficacy of bioactive glass was significant in both primary maxillary and mandibular first molar and second molar proving it to work best for all types of root anatomy in mixed dentition population. Superior clinical and microbiological results were found when bioactive glass was used as intracanal medicament in both intraoral and extra oral abscess cases in primary molars.

**Conclusions:** Bioactive glass; intracanal medicament; primary dentition; primary molar; mixed dentition.

**Keywords:** Elastic modulus, flexural strength, provisional restorative materials

### Introduction

The use of intracanal medicaments have become inevitable in pulp therapy of primary teeth due to their complex root anatomy<sup>[1]</sup>. Mostly, the organic residues and bacteria located in the dentinal tubules cannot be sufficiently cleaned even after meticulous mechanical procedures. Antimicrobial irrigants will definitely eradicate the pathogens up to certain limit. But the probability of the remaining bacteria to multiply enormously between appointments, often reaching the same level that it was at the start of the previous procedure is more in cases if the canal is not dressed with a disinfectant between visits<sup>[2]</sup>. However, no single medicament has proved capable of meeting all requirements and therefore a vast range of materials have been tried.

Hence, this present study is aimed to evaluate the efficacy of Bioactive glass as an intracanal medicament in primary molars. Bioglass, a silica-based melt-derived glass has been widely used in medicine and dentistry. Hench at the University of Florida introduced the first bioactive glass in 1969 [3]. Its composition of 46.1 mol. % Silicon dioxide (SiO<sub>2</sub>), 24.4mol. % Sodium oxide (Na<sub>2</sub>O), 26.9 mol.% Calcium oxide (CaO) and 2.6 mol.% Phosphorus pent oxide (P<sub>2</sub>O<sub>5</sub>) [4]. These glasses are being used as particulates or monolithic shapes and porous or dense constructs in different applications such as regeneration of bone, remineralization and hypersensitivity treatment. Additionally, this active biomaterial has antimicrobial and anti-inflammatory effects.[5] The objective of the study is to assess the reduction in bacterial count when Bioactive glass, 1% Chlorhexidine gel with Calcium hydroxide powder and 1% Chlorhexidine gel used as an intracanal medicament in primary molars.

## Methods

The *in vivo* study was carried out in the Department of Pediatric and Preventive Dentistry and the microbiological assay was performed in Department of Microbiology, Krishnadevaraya College of Dental Sciences and Research centre in children of 4- 11 years of both genders requiring multiple visit Pulpectomy procedures for their primary teeth for 48 subjects who met *al.l* of the inclusion criteria.

Selection criteria included subjects who were indicated for multiversity Pulpectomy with minimal mobility, minimal bone loss with restorable crown and minimal root resorption or bone resorption seen radiographically. Subjects who have any one of the following diagnosis in their primary maxillary / mandibular molars with chronic pulpitis, Acute exacerbation of chronic pulpitis, Chronic abscess, Acute exacerbation of chronic abscess were selected for the study.

Subjects who were treated with antibiotics within 3 months, subjects giving history of systemic diseases, subjects having pathologic mobility of primary molars, gross peri-radicular bone loss of primary molars, external resorption and internal resorption with perforation and retreatment cases were excluded from the study.

The study purposes were fully explained to parents/guardians, who signed a written informed consent form after getting the verbal consent from the children. The research protocol was received and approved by Institutional Research Ethical Board.

## Materials tested include

- i. Bioactive glass (NOVABONE - PERIOGLAS®) – particle size 90-710 µm
- ii. 1% Chlorhexidine gel (HEXIGEL®) + Calcium hydroxide powder (DP – Extra pure)
- iii. 1% Chlorhexidine gel (HEXIGEL®)

The subjects were randomly divided into 3 experimental groups.

**Group 1:** Bioactive glass as intracanal medicament

**Group 2:** 1% Chlorhexidine gel + Calcium hydroxide powder as intracanal medicament

**Group 3:** 1% Chlorhexidine gel as intracanal medicament.

A standardized protocol, as described below, was followed for the Pulpectomy procedure and collection of bacterial cultures. First visit, Diagnostic pre-operative IOPAR was obtained for each tooth. After case selection local anaesthesia was administered and the tooth was isolated using rubber dam. Gross caries removal was done using a sterile round bur (BR-

46) attached to an airtor hand piece.

The operating tooth was disinfected with 30% Hydrogen peroxide and 10% Iodine tincture, followed by inactivation of the above solutions by 5% Sodium thiosulfate solution as suggested by Moller *et al.* [6], and sample S0 was taken using a sterile paper point size 15 held in place for 60 seconds, further transported to microbiological lab in an Eppendorf tube containing 1 ml of sterile Thioglycolate broth. Access opening; de-roofing was done followed by Working length estimation by radiographic method. A sterile 15 size paper point was introduced into the length of the canal as determined radiographically for pre – procedural microbiological sampling (S1). The paper point of size 15 was kept in place for 60 seconds and immediately transferred to Eppendorf tube containing 1 ml of sterile Thioglycolate broth. Copious irrigation with saline was done and cleaning and shaping was done using H- file and K- file systems starting 1 mm above the apex up to size 40. The canals were dried with sterile paper points and medicament was placed inside the canal by loading it in a 2ml syringe and a double seal was done with Cavit G and Glass Ionomer Cement (Type IX). The patient was recalled after 5- 7 days for all group patients.

Bioactive glass is marketed in the particle size of 90-710 µm. The particle size was reduced to facilitate its usage as an intracanal medicament. Hence, the crystalline powder was transferred into a sterile ceramic mortar and pestle, crushed into smaller size and then passed through a sieve having mesh size of 45 µm. The final particle size was 45 µm, suitable to be used as a medicament in Group 1 individuals. It was carried in a tuberculin syringe and condensed into the canal using plugger.

Second visit, after 5 – 7 days, with rubber dam isolation, the double seal was removed. The medicament placed inside the canal was flushed with saline. After copious irrigation with saline, canals will be dried and S2 sample will be taken as follows: Sterile paper points of size 15 was placed; held for 60 seconds and transferred into 1 ml of sterile Thioglycolate broth to determine the CFU/MI [FIGURE 1]

Microbiological samples were incubated for 60 minutes and shaken vigorously in a vortex mixture for 30 seconds. Under strict conditions of asepsis, from 1ml of the sample in the Eppendorf tube, 50 µl of the undiluted sample was taken and were inoculated in the culture media (Hi Media- Sheep blood agar medium) and were spread uniformly using L spreaders. The plates were then kept in the incubator for 24 hours and were counted for Colony Forming Units/ml (CFU/mL). [FIGURE 2]

After the microbiological sampling, the Pulpectomy procedure was completed by obturation followed by stainless steel crown.

Statistical analysis was carried out using Microsoft Excel and Software Package for Social Sciences (SPSS Inc, Chicago v 18.5) software were used for data entry and analysis respectively. Normality test (Shapiro Wilk test) revealed that few parameters in the study are not normally distributed, so comparison between the groups was carried out using nonparametric test like Kruskal Wallis test, One- way Anova and Chi-Square test.

## Result

A total of 48 teeth were studied. They included three groups of 16 teeth each. Group I was medicated with bioactive glass, Group II with 1% Chlorhexidine gel + Calcium hydroxide powder, and Group III with 1% Chlorhexidine gel.

Root canal samples from each tooth were obtained thrice,

wherein 'S0' and 'S1' was taken during 1<sup>st</sup> visit and 'S2' was taken during 2<sup>nd</sup> visit, 5-7 days apart.

It was revealed that the operating tooth had minimal / reduced number of bacterial colonies [Table 1]. This was achieved by sterilizing the operating tooth using 30% Hydrogen peroxide, 10% Iodine tincture and 5% Sodium thiosulphate and it was highly evident when compared with the values of S1 [Table 2]. Similarly, mean S2 values were plotted in the table [Table 3].

The mean percentage reduction in the bacterial count from before and after the placement of the medicament (S1 - S2 value) among the study groups were plotted in a table and graph and the difference obtained was statistically significant (i.e) p value of 0.001 achieved [Table 4 and Graph 1] in which Bioactive glass showed 75.7% of bacterial reduction.

Result obtained from this study proves the antimicrobial efficacy of bioactive glass in root canal sterilisation.

Comparison of three groups is presented in [Figure 3].

## Discussion

Root canal treatment can be performed either in single or multiple visits [7]. Irrespective of the preferred approach, it should be realized that disinfection of a root canal system takes time [8]. The difficulties of complete root canal disinfection and the liability to reinfection still remain and render the prognosis less favourable in chronically infected teeth. Hence, the importance of chemical means of disinfection has aroused to accomplish the complete sterilization of canal and to improve the treatment prognosis [9]. Consequently, treatment of teeth with furcal lesions in two visits using an antiseptic root canal interim dressing remains the gold standard. The use of an intracanal medication is that it could potentially disrupt the nutritional interrelationships among the microbes by eliminating some bacteria that are essential for the growth of other bacteria or leaving some bacteria intact whose presence would inhibit the growth of other bacteria [10]. One possible disadvantage of this approach, however, is that the root canal system can be contaminated by resident or transient oral microbiota during the multi visit period due to leaking temporary restorations [11].

Various intracanal medicaments have been reported in the literature. They include Essential oils, Phenolic compounds like Formaldehyde, Paraformaldehyde, and Glutaraldehyde, Halogens like Iodine, Antibiotics like Penicillin, Metronidazole, Cephalosporins, and Combinations of various antibiotics, Calcium hydroxide, Chlorhexidine and various plant extracts [12]. Despite conflicting claims, no medicament appears to be ideal and significant variability exists in clinical dental practice regarding their use so newer materials are being tried to overcome disadvantages of the existing materials [13].

In this present study antibacterial efficacy of Bioactive glass, 1% chlorhexidine gluconate and calcium hydroxide combination and 1% chlorhexidine gluconate were studied.

Calcium hydroxide has been used in root canal treatments to obtain disinfection of the canals. However, literature reviews have concluded that it is less effective, even resistance against *Enterococcus faecalis*. Also, it shows limited action against facultative anaerobes and *Candida albicans* [14]. Hence it was considered that addition of vehicles or other agents might contribute to the antimicrobial effect of calcium hydroxide. Many studies revealed that the effect of Calcium hydroxide in combination with Chlorhexidine showed higher antimicrobial effect against facultative anaerobes and *Candida* species in comparison with plain Calcium hydroxide.

Chlorhexidine has been widely used as an intracanal medicament because of its wide antimicrobial spectrum and it is effective against both Gram-positive and Gram-negative bacteria as well as yeasts. It acts by electrostatic interaction as it is positively charged and the bacterial wall is negatively charged, allowing bacterial cytoplasm coagulation and results in cell death [15]. Due to the substantively potential it is successful in treatment of persistent infection and endodontic failure cases. But it has a potential disadvantage that it does not act as a barrier against microbial recolonization and does not detoxify the endotoxins produced by the microbes [16].

Bioactive glass has antimicrobial and anti-inflammatory effects [5]. The antimicrobial potential is because of its ability to raise the pH in aqueous suspension. These high pH levels are not well tolerated by bacterial cells. The broad-spectrum antimicrobial effect of bioactive glass (BAGs) on different oral microorganisms has been reported which justifies its use as an intracanal medicament in pulp therapy [17].

The results of this study reveals that the mean percentage of reduction of bacterial count from S1-S2 (Before and after placement of medicament) with Group 1: Bioactive glass was 75.7%, with Group 2: 1% Chlorhexidine gluconate and calcium hydroxide was 52% and Group 3: 1% Chlorhexidine gluconate was 28.6%, revealing that Bioactive glass has the superior antibacterial efficacy when compared with other groups.

Our study results are in accordance with Goel *et al.* (2015) [17], who concluded that in *in vivo* environment the antimicrobial efficacy of Bioactive glass was better when compared to 1% Chlorhexidine.

But Krithikadatta *et al.* (2007) [18] and Atila-Pektas *et al.* (2013) [19], conducted *in vitro* studies and concluded results in contrary to this study. However, the important fact to be noted in these two studies is that both the studies were conducted in an *in vitro* environment and on permanent teeth.

Efficacy of usage of Chlorhexidine gluconate gel alone and its combination with calcium hydroxide had been a matter of dispute in the past study. While some studies revealed its superior efficacy in combination with Calcium hydroxide, some studies did not yield to it. Likewise, in this study, we found that the Combination of Chlorhexidine gluconate with Calcium hydroxide has given more efficient bacterial colony reduction when compared to Chlorhexidine alone.

These results are in accordance with Oncag *et al.* (2006) [10], who concluded that 1% Chlorhexidine gluconate gel with calcium hydroxide was most effective intracanal medicament against *E. faecalis* and with Sinha *et al.*, (2013) [13], who compared in *in vivo* conditions and concluded that although not statistically significant Chlorhexidine gluconate gel with Calcium hydroxide showed better antimicrobial effect when compared other medicaments.

These results clearly explain the antibacterial activity of bioactive glass when compared to the standard intracanal medicaments like Chlorhexidine and Calcium hydroxide.

It is believed that the antimicrobial potential of bioactive glass is largely a function of their ability to raise the pH in aqueous suspension. These high pH levels are not well tolerated by either bacteria or host cells. The other mechanism is that the dissolution of Silica from the glass takes place and this exerts an indirect effect by promoting calcium and phosphate precipitations which interferes with the cellular integrity of bacteria. Silica acts as a surfactant at solid - liquid interfaces and may thus directly inhibit the bacterial viability. Thus, exhibiting dual mechanism of antibacterial activity [20].

The probable reasons for less efficiency of Chlorhexidine

alone and its combination with Calcium hydroxide as compared to Bioactive glass may be due to inhibition of Chlorhexidine and calcium hydroxide activity by organic part of dentin - Type 1 collagen, acid proteins, glycoproteins, periapical exudate, and dead microbial cells present in the dentinal tubules whereas the action of Bioactive glass is enhanced in the presence of increased pH, dentin, and silica [21, 22].

Also, the mean percentage reduction of bacterial count (S1-S2) among the primary 1st molar (72.6%) and 2nd molar

(78.7%) in Bioactive glass group was comparable proving its efficacy in both the molars which have characteristic morphologically different root anatomy [Graph 2]. In addition to the excellent microbial success, Bioactive glass also yielded best clinical results when used on intraoral and extra oral abscess cases, with significant reduction in the swelling size in the interim period.

To conclude our present study, bioactive glass can be considered as one of the best emerging alternatives for the conventional intracanal medicaments available in dentistry.

**Table 1:** Comparison of Mean S0 bacterial count (CFU/ml × 10<sup>3</sup>) among Study Groups

Group	N	Mean	SD	Median	Min.	Max.	Chi-square*	P value
Group 1	16	134.5	66.161	122.0	40	304	0.025	0.988
Group 2	16	148.5	109.971	140.0	12	384		
Group 3	16	139.5	97.026	136.0	20	440		

Comparison of Mean S0 bacterial count among Study Groups

**Table 2:** Comparison of Mean S1 bacterial count (CFU/ml × 10<sup>3</sup>) among Study group

Group	N	Mean	SD	Median	Min.	Max.	ChiSquare*	P value
Group 1	16	5,340.30	4,244.106	4,164.0	600	13608	5.517	0.063
Group 2	16	2,085.50	1,735.447	1,608.0	264	7156		
Group 3	16	3,332.50	3,103.485	2,468.0	428	10572		

Comparison of Mean S1 bacterial count among Study Groups

**Table 3:** Comparison of Mean S2 bacterial count (CFU/ml × 10<sup>3</sup>) among Study groups

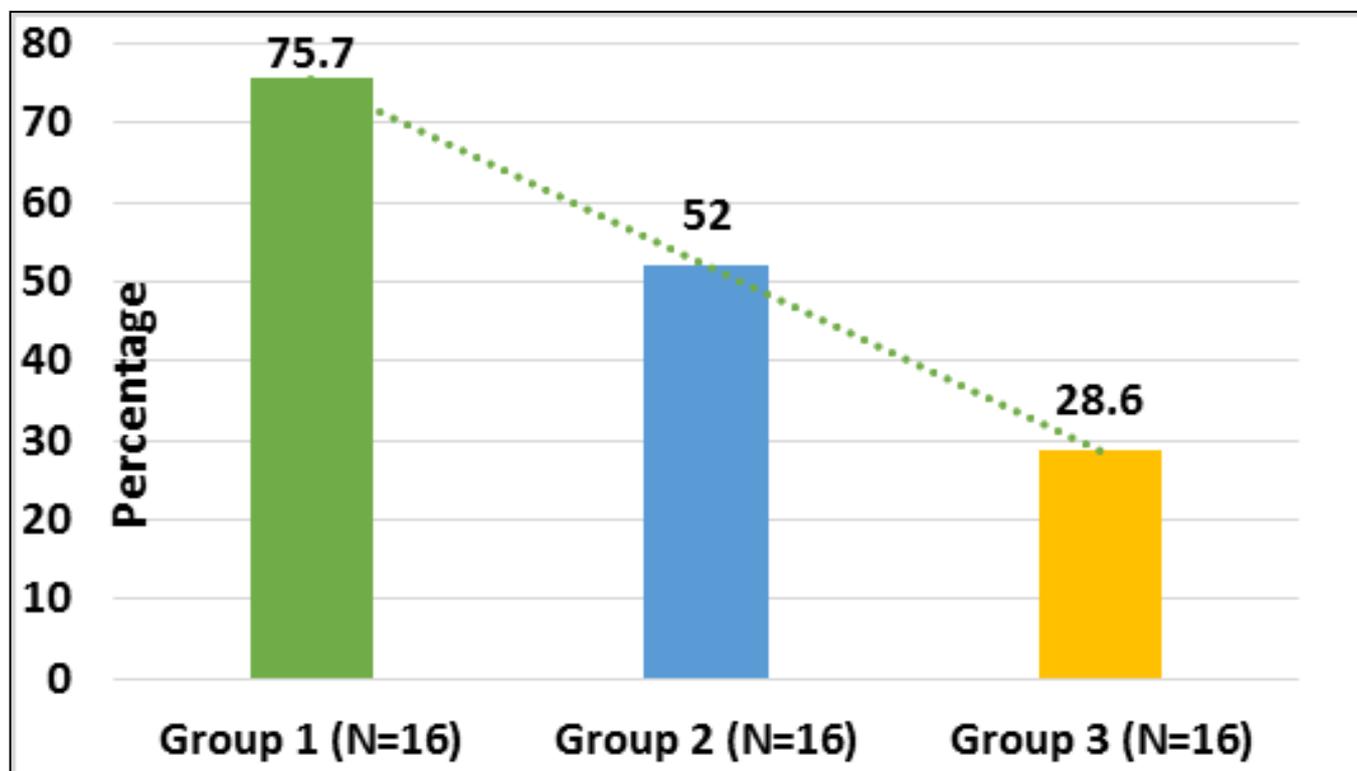
Group	N	Mean	SD	Median	Min.	Max.	Chi-square*	P value
Group 1	16	1,332.8	1669.003	400.0	28	5188	5.159	0.076
Group 2	16	964.00	938.690	450.0	136	3560		
Group 3	16	2,416.00	2281.260	1,898.0	100	7920		

Comparison of Mean S2 bacterial count among Study Groups

**Table 4:** Comparison of the mean percentage reduction of bacterial count among the Study Groups

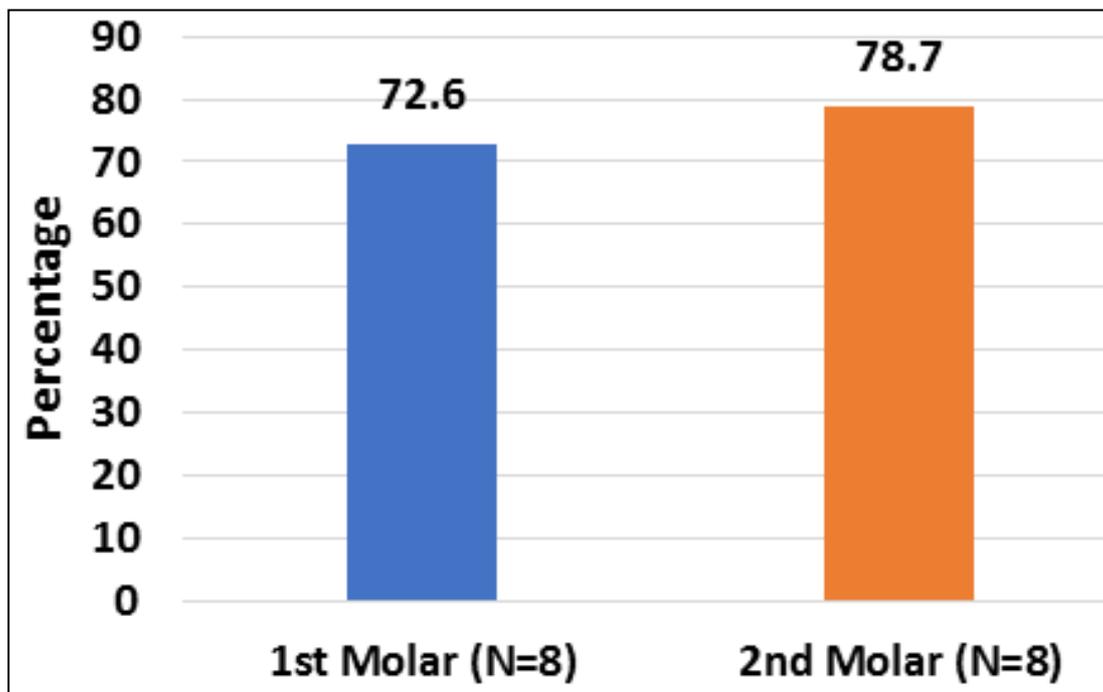
Group	N	Mean	SD	Median	Min.	Max.	F value	P value
Group 1	16	75.7	16.299	69.5	56.6	99.5	29.092	<0.001
Group 2	16	52.0	17.550	47.6	33.3	88.6		
Group 3	16	28.6	18.451	23.4	8.5	93.9		

Comparison of Mean S1-S2 bacterial count among study groups. P-value of significance obtained



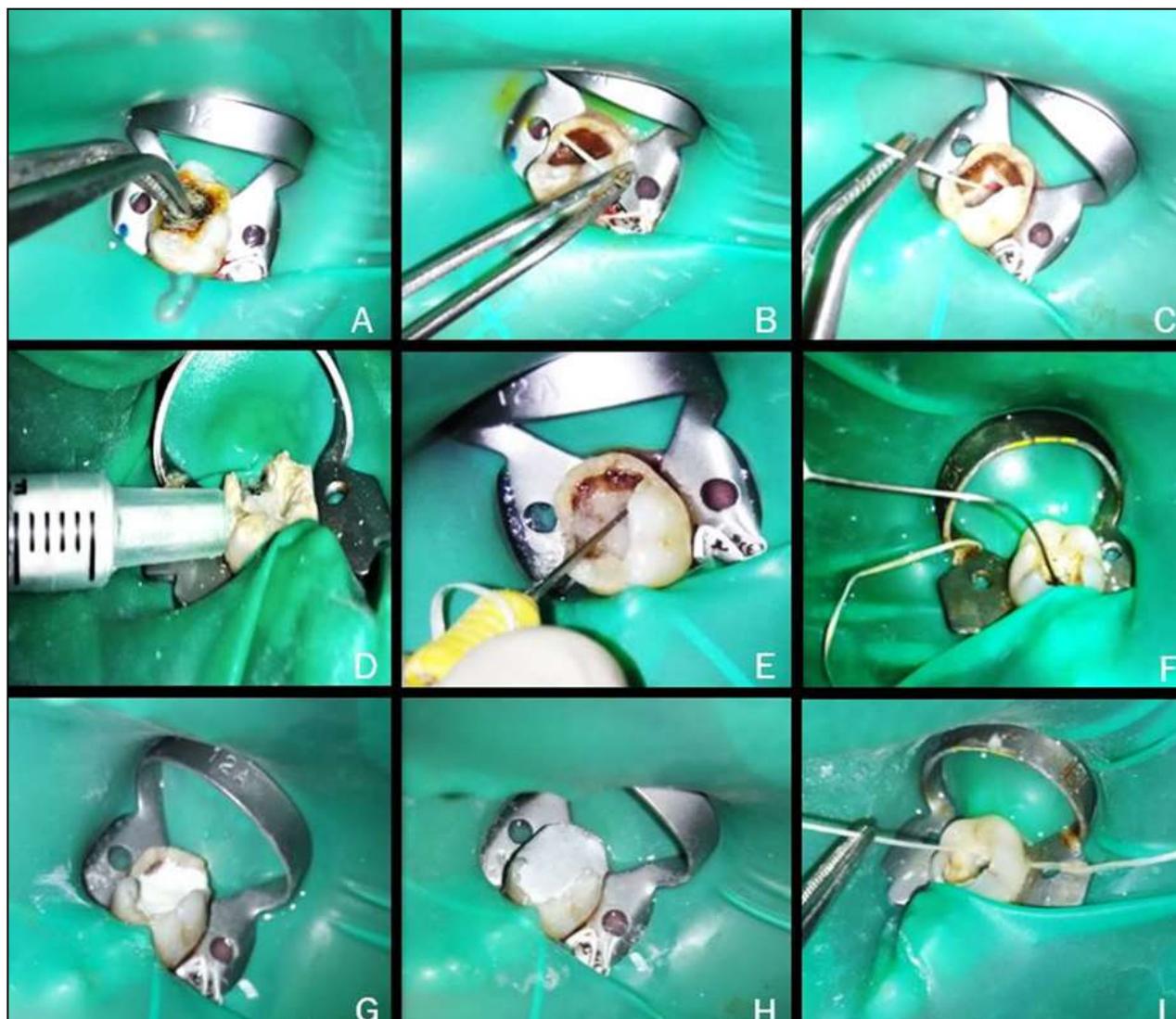
The mean comparison of percentage reduction in the bacterial count from before and after the placement of the medicament (S1-S2 value) among the study groups was statistically significant

**Graph 1:** Comparison of the mean percentage reduction of bacterial count among the Study Groups

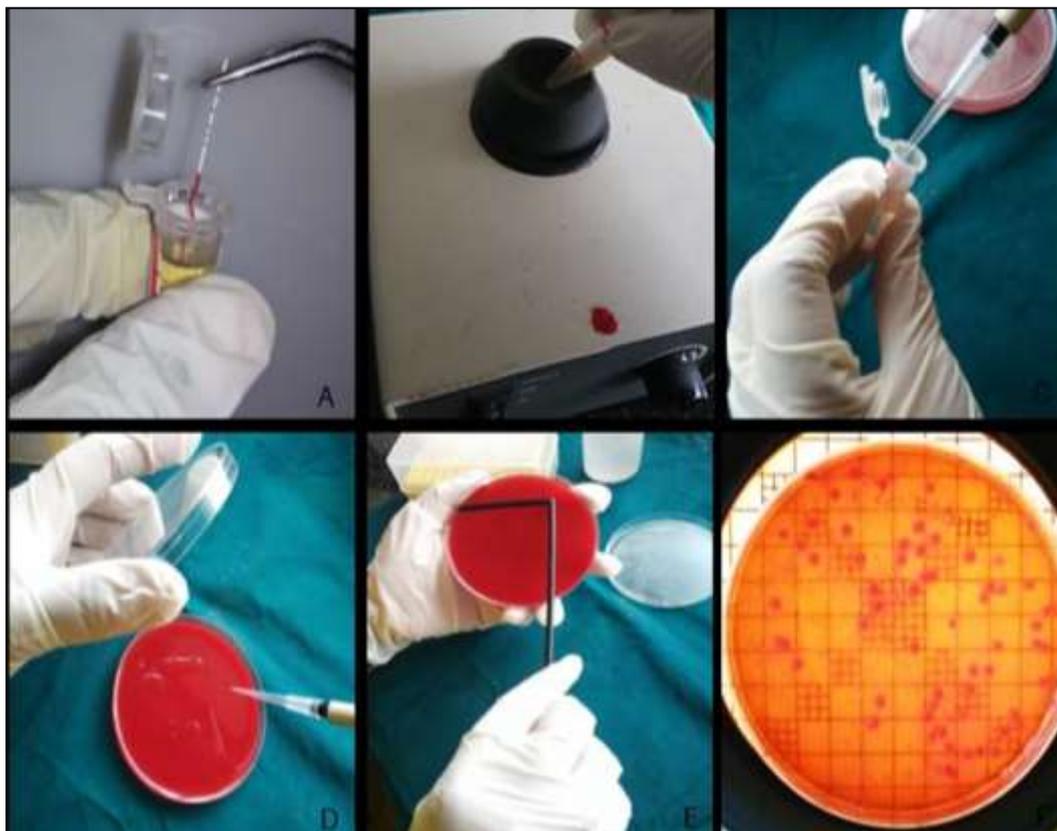


Comparison of mean (S1- S2) in 1<sup>st</sup> and 2<sup>nd</sup> molar of Group I treated with Bioactive glass

**Graph 2:** Mean percentage reduction of bacterial count (S1-S2) in 1<sup>st</sup> and 2<sup>nd</sup> molar of Group I treated with bioactive glass



**Fig 1:** Sterilization of the cavity (A); Sample S0 taken (B); Sample S1 taken after working length determination (C); Intracanal medicaments placed- Bioactive glass carried using tuberculin syringe and condensed using hand plugger (D&E); Group 2 & 3 medicaments placed into canal using 2ml syringe (F); Double seal done with Cavit G and Glass Ionomer Cement (G&H); Sample S2 collected after 5-7 days (I)



**Fig 2:** Transferring the paper point into broth (A); Vortexing of the sample for 30 seconds (B); 50 µl of sample taken (C); Inoculation and spreading of sample on Sheep blood agar (D & E); Colony Forming Units/ml counted using digital colony counter (F)

GROUPS/ SAMPLES	SAMPLE- S0	SAMPLE - S1	SAMPLE - S2
GROUP 1 BIOACTIVE GLASS			
GROUP 2 1% CHLORHEXIDINE GLUCONATE AND CALCIUM HYDROXIDE			
GROUP 3 1% CHLORHEXIDINE GLUCONATE			

**Fig 3:** Comparison of colony count

**Conclusion**

Based on the present study, in case of multiple visit lumpectomies *in vivo*:

- Within the limitations of this study, the antibacterial efficacy of Bioactive glass was higher (75.7%) when compared to combination of 1% Chlorhexidine gluconate

and Calcium hydroxide (52%) and 1% Chlorhexidine gluconate alone (28.6%).

- Use of Bioactive glass in deciduous first and second molars (both maxillary and mandibular molars), in mixed dentition age group of 4-11years (mean = 7years) resulted in statistically significant reduction ( $P < 0.01$ ) in Total microbiological count, when placed as intracanal medicament for 5-7 days.
- Bioactive glass showed superior clinical result when used as intracanal medicament in both intraoral and extra oral abscess cases in primary molars.

Though bioactive glass is little towards the expensive side when compared to others, the results of this study are highly encouraging to put it into practice. We can safely conclude that bioactive glass is the best emerging alternative for the conventional intracanal medicaments available in dentistry.

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