Original Research Paper

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An In Vitro Study Evaluating Glass Ionomer Cement Antibacterial Activity, Shear Bond Strength, and Compressive Strength with the Incorporation of Clove Oil

Abstract:

Objective: This in vitro study aimed to evaluate and compare the antibacterial activity, shear bond strength, and compressive strength of conventional glass ionomer cement (GIC) and GIC modified with clove oil.

Materials and Methods: Eighty samples were prepared and divided into two groups: conventional GIC (control) and clove oil-modified GIC. The antibacterial efficacy was assessed against Streptococcus mutans and Lactobacillus acidophilus using the minimum inhibitory concentration (MIC) method. Shear bond strength and compressive strength were evaluated using a Universal Testing Machine following standardized protocols. Statistical analysis was performed using independent t-tests, with significance set at $p \leq 0.05$.

Results: The clove oil-modified GIC demonstrated significantly lower MIC values, indicating enhanced antibacterial activity compared to the control group ($p < 0.001$). Additionally, the modified GIC showed significantly higher shear bond strength (10.93 ± 0.47 MPa) and compressive strength (182.68 ± 2.90 MPa) compared to the control group (8.58 ± 0.56 MPa and 160.60 ± 7.98 MPa, respectively), with p values of 0.001 and 0.002.

Conclusion: Incorporating clove oil into GIC significantly improves its antibacterial properties, shear bond strength, and compressive strength. This modification shows promise for enhancing the clinical effectiveness of GIC in caries-prone patients, although further in vivo studies are recommended to assess its long-term performance and clinical applicability.

Key-words: .....

Introduction:

Children's health and wellbeing may suffer as a result of early childhood caries, a chronic illness that is common among young children. It's not fatal, but it's still a serious problem. Scientific research is continuously focused on finding the best strategies for treating and preventing dental caries. As part of this endeavor, minimal intervention dentistry—a contemporary dental technique—is progressively gaining awareness [1]. Preserving the greatest amount of the patient's original tooth structure is the main goal. One prominent example of how minimum intervention dentistry applies this idea is atraumatic restorative treatment (ART) [2]. As part of an alternate restorative procedure, soft, totally demineralized carious tooth tissue is removed using hand tools [3]. Following the removal of the carious tissue, an adhesive dental substance, like glass ionomer cement (GIC), is used to repair the cavity. GICs are known for their biocompatibility, capacity to release fluoride ions gradually, and propensity to stick to enamel and dentin [4].

It is noteworthy to add that although rotary burs have been shown to be more successful at removing germs than manual devices, some contaminated dentin may still be present in cavities treated with ART. Because cariogenic bacteria can

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live beneath the GIC repair and continue to grow for up to two years, secondary caries may result [5]. Previous studies indicate that the fluoride produced by GIC is not strong enough to prevent bacterial deterioration over a lengthy period of time [6]. Due of this shortcoming, antibacterial agents will be added to GIC. The existing GIC has been altered in an attempt to build an effective one since previous research on similar adjustments resulted in changes in physical characteristics.

The state of affairs is changing globally, with a move toward the use of botanical items in dentistry—a practice known as photo-dentistry [7]. The essential oil of cloves (Syzygiumaromaticum), which is extracted from the fragrant flower buds of the Myrtaceae family tree, is used to treat dental crises by relieving pain. Cloves have been used for more than 2,000 years in India and China for their use as spices, to prevent tooth decay, and to treat bad breath [8]. Cloves are particularly effective against germs that are connected to periodontal disease, tooth problems, and other microbial hazards. Cloves' main ingredient, eugenol, is included into tooth restorative materials and is effective against mouth bacteria. Because of its effects on gingivitis, plaque, and oral flora, researchers are looking into using cloves as a natural way to maintain dental health. Studies have also shown that clove oil demonstrates powerful antibacterial action [9]

The existing body of research on the use of neem in GIC for healing purposes is deficient in evidence. There is little study on their usage as restorative materials, despite established literature demonstrating their efficacy in mouthwash and toothpaste. Thus, the goal of this study is to compare modified GIC with clove oil to conventional GIC in order to evaluate the antibacterial efficacy and compressive strength of modified GIC. According to the null hypothesis, standard GIC and GIC modified with clove oil are identical.

Methodology:

The necessary sample size was ascertained using the GI Power sample size calculator. Based on the computation, 80 samples would be needed to provide a power of 0.95 (95% confidence interval) with an effect size of 0.25 for each metric that includes shear bond strength, compressive strength and antibacterial activity.

This study employs an experimental in vitro intergroup comparative design to assess and compare the antibacterial activity, shear bond strength and compressive strength of clove oil incorporated GIC. The research will be conducted in the Department of Paediatric and Preventive Dentistry, Maharana Pratap College of Dentistry & Research Centre, Gwalior, Madhya Pradesh, India adhering to standardized protocols and procedures. In this study, type IX GIC (GC Corporation) was employed. To create the experimental cement, clove oil was extracted using standardized technique as discussed later and incorporated to the GIC powder. The experimental groups consisted of group I (control group with conventional GIC), group II (clove oil incorporated GIC).

After that, the disk-shaped specimens will be contrasted with unaltered GIC (control). The minimum inhibitory concentration (MIC) test against Lactobacillus and Streptococcus mutans was used to evaluate the efficacy of the antibiotics. A Universal Testing Machine was used to measure shear bond strength and compressive strength, and the crosshead speed was adjusted to 0.5 mm per minute.

The experiment made use of five-day-dried clove buds. Glassware was cleaned, given a distilled water rinse, and then allowed to dry at 70°C. 100 mL of distilled water and one gram of cloves will be combined, then cooked for fifteen minutes at 60 to 70°C on a heating mantle. Following filtering using Whatman No. 1 filter paper (Whatman Plc, Maidstone, UK), 80 milliliters of the filtrate will be collected and placed in an individual Erlenmeyer flask separately. After filtering, this extract was reduced to a 5 ml amount.

To ensure complete blending, the material was first stirred by hand and then vibrated for five minutes. Each group of experiments had a set of disk-shaped specimens that measured 6 mm in diameter and 2 mm in height. In order to create a paste-like texture, the powder and liquid had to be mixed and then poured into a silicone mold. The specimens will be taken out of the mold and put in containers with the proper labels after a 30-minute wait. They will be then kept for 24 hours at 37°C in an incubator before being tested to determine their antibacterial capabilities. A solitary, skilled operator processed each sample at a room temperature of 23°C.

Antibacterial efficacy:

The Department of Microbiology provided strains of Lactobacillus acidophilus and Streptococcus mutans, which will be then grown on Mueller-Hinton agar and then moved to Mueller-Hinton broth. Following a 24-hour incubation period at 37°C, 1.5 × 108 CFU of bacterial suspensions will be determined. The antibacterial activity of both modified and unmodified GIC was assessed using standard strains of Lactobacillus and Streptococcus mutans. Every group had 40 specimens. First, we made Mueller-Hinton agar broth and filled each group's well with 200 µL of it. The wells will be

filled with bacterial suspensions of Lactobacillus acidophilus and Streptococcus mutans, each having around 5 × 105 CFU/mL. Assays measuring the minimal inhibitory concentration (MIC) will be conducted for the modified group. Throughout the incubation phase, the samples will be kept in appropriate settings for one to four hours at a time. By detecting absorbance at a wavelength of 540 nm, an enzyme-linked immunosorbent assay reader was used to determine the percentage of cell death at particular time points.

Compressive strength evaluation:

The compressive strength evaluation followed ISO 9917-1:2007 standards, with 40 specimens prepared using cylindrical molds for each group. The specimens will be allowed to go through the setting process for an hour after the materials will be put into the molds and a smooth surface was checked. They will be then kept for a full day in a deionized water immersion. The research did not include any specimens that will be discovered to have flaws. The specimens will be placed into the Instron Electro Plus E3000 Universal Testing Machine vertically. Until the specimens reached the fracture point, a compressive force was applied at a rate of 0.5 mm/minute along their expanded axis. The compressive strength in mega pascals (MPa) was determined by taking note of the greatest force at which the specimens broke. Using a 5 kN load cell, compressive force was applied as part of the testing methodology.

Shear bond strength:

Zwick GmbH, Ulm, Germany's Z020 universal testing equipment was used to measure the shear bond strength. The teeth will be positioned such that the applied force was perpendicular to the bracket, and the shear force was transferred to the bracket at a crosshead speed of 1 mm/minute. Mega pascals (MPa) are the units used to measure the bond strength and the force needed to shear the bracket. The results of this investigation will be contrasted with Reynolds' indicated bond strength range of 5.9–7.8 Mpa.

The gathered information was input into a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA), and SPSS version 24 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. The mean MIC values were ascertained and descriptive analysis was made. An Independent student 't' test was used to compare the compressive strength and shear bond strength across the groups. P-values ≤ 0.05 were used as the significance threshold, and 95% confidence intervals were taken into account.

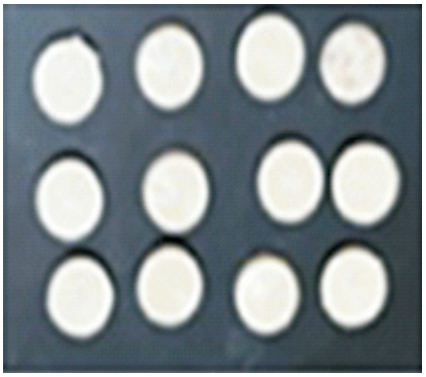


Figure 1: Preparation of sample

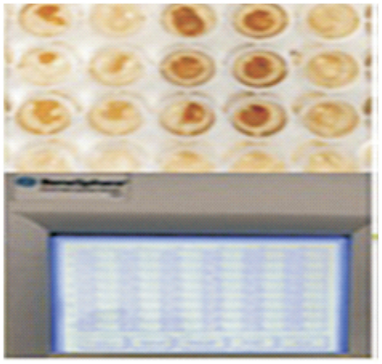


Figure 2: ELISA

Figure 3: Cylindrical moulds

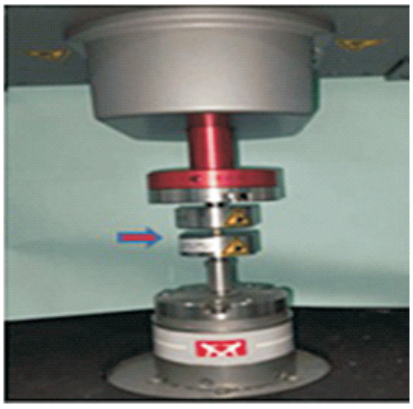


Figure 4: Electro Plus E3000

Results:

The obtained data was summarized into a data sheet and sent for statistical analysis. The MIC of the Streptococcus mutans and Lactobacillus acidophilus was compared between the control group and the clove induced GIC group. Similarly, the shear bond strength and compressive strength was compared between the groups. On analyzing the data obtained and statistical analysis, it was found that the MIC of the streptococcus and lactobacillus was least among the clove induced GIC group than control group, which denotes the higher antimicrobial efficacy. The mean MIC of the streptococcus was 0.25(0.03) and MIC of the lactobacillus was 0.21(0.02) among the clove modified group. The mean MIC of the streptococcus was 0.52(0.02) and MIC of the lactobacillus was 0.47(0.04) among the control group The difference was found to be highly significant with p value of <0.001.[Table 1][Graph 1]

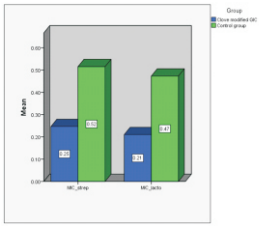
Table 1: The comparison of the antibacterial efficacy based on the MIC

Group	MICstreptococcus		MIClactobacillus	
	Mean	Standard Deviation	Mean	Standard Deviation
Clove modified GIC	.25	.03	.21	.02
Control group	.52	.02	.47	.04
F value	210.33		140.74	
P value	<0.001**		<0.001**	

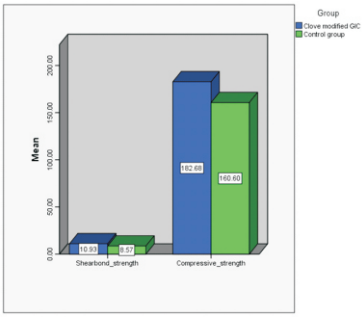
When the shear bond and the compressive strength was analyzed and compared. It was found that the strengths were significantly higher in the clove modified GIC groups, than the control group. The mean Shear bond strength of control group was 8.58(0.56) Mpa and Clove modified GIC group was 10.93(0.47) Mpa. The difference was significant with P value of 0.001. The mean Compressive strength of the control group was 160.6(7.98) Mpa and Clove modified GIC group was 182.68(2.90) Mpa. The difference was significant with P value of 0.002. [Table 2][Graph 2]

Table 2: The comparison of the shear bond and compressive strength based on the MIC

Group	Shear bond strength		Compressive strength	
	Mean	Standard Deviation	Mean	Standard Deviation
Clove modified GIC	10.93	.47	182.68	2.90
Control group	8.58	.56	160.60	7.98
F value	41.95		27.004	
P value	0.001		0.002	



Graph 1: The comparison of the shear bond and compressive strength antibacterial efficacy among groups



Graph 2: The comparison of the among groups shear bond and compressive strength among groups

Discussion:

Dental caries is a common dental disease that is mostly brought on by cariogenic bacteria, of which Streptococcus mutans is a major component. The glucosyltransferase enzyme, which Streptococcus mutans produces, aids in the conversion of sucrose into water-insoluble glucan, which sticks to dental enamel. The colonization of Streptococcus mutans and other Streptococci species is facilitated by this sticky layer. Afterwards, by the fermentation of sucrose, acidogenic bacteria such as Lactobacillus species create an acidic environment [10]. When acidogenic and acid uric bacteria combine to generate dental plaque, acid build up and mineral decalcification result in localized tooth damage. To address caries development and spread, it is crucial to comprehend the antibacterial activity of recently synthesized restorative materials against these fundamental microorganisms.

The Lactobacillus acidophilus and Streptococcus mutans minimum inhibitory concentrations (MICs) were examined in the clove-induced GIC group and the control group. Comparing the compressive and shear bond strengths between the groups was also done. The results showed that the clove-induced GIC group had lower minimal inhibitory concentrations (MICs) for lactobacillus and streptococcus than the control group, indicating better antimicrobial activity. when the compressive strength and the shear bond were examined and contrasted. It was discovered that the clove-modified GIC groups' strengths were noticeably higher than those of the control group.

Since ancient times, cloves (Syzygiumaromaticum), a highly valued spice, have been utilized for a variety of medical purposes as well as food preservation. Cloves have been shown to be effective against a variety of germs, including those linked to periodontal disease, dental caries, and other

infections [8].Because clove disrupts bacterial cell membranes, it has shown antibacterial activity against common oral infections such as Streptococcus mutans [11]. Clove oil's antibacterial efficacy against Lactobacillus acidophilus, Streptococcus salivarius, and Streptococcus sanguis was confirmed by the Voleti et al. research [12]. Clove and clove bud oil were discovered to be viable antibacterial agents for the treatment of dental caries in Aneja and Joshi's study [13]. In contrast to our results, Gupta et al.'s comparative investigation of clove oil and clove extract showed a greater zone of inhibition in clove oil. Clove oil's inability to chemically adhere to the polyalkenoate matrix and glass's potential to impede the material's setting process might be the cause of this discrepancy in our investigation [14].

According to ISO 9917 (2007), it is imperative to evaluate the compressive strength of dental materials.This higher concentration of clove extract, backed by the phytochemical constituents that increase compressive strength, may be responsible for Group with clove-modified GIC's elevated compressive strength. This is consistent with a study by Singer et al. [15] that found that incorporating a higher concentration of plant extract with GIC produced a good compressive strength. In a similar study, the group with the incorporation of clove extract into GIC has demonstrated a substantial enhancement inantibacterial activity against cariespromoting organisms and a significant increase in compressivestrength, which is in concurrence with the current study [16]

Consequently, patients at high caries risk benefit most from clove-modified GIC's increased antibacterial activity and compressive strength, which also helps to prevent secondary caries and improves the drug's therapeutic effectiveness. This study's intrinsic shortcoming stems from its failure to account for intraoral factors, including masticatory strains, moisture content, and possible operator differences. Nevertheless, in order to assess its economic and practical viability and support its usage in clinical applications, more research in the fields of molecular chemistry and clinical settings is advised.

Conclusion:

On the basis of the results obtained it can be clearly concluded that the clove induced GIC have better antibacterial efficacy, increased shear bond strength, and increased compressive strength, when compared to conventional GIC. To evaluate and investigate the long-term stability and clinical usefulness of this modified GIC in preventing and fighting dental caries, more investigation and clinical studies are necessary.

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