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RESEARCH ARTICLE
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ANTIMICROBIAL EFFECTS OF SPIDER SILK INCORPORATION IN GLASS IONOMER CEMENT

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ABSTRACT

Objective: An agar plate diffusion test was used to assess the bactericidal activity of GIC combined with 5, 10, and 15% spider silk protein.
Design of the study: An-in vitro experiment.
Study Setting: Sardar Begum Dental College in Pakistan, Department of Science in Dental Materials.
Methodology: The specimen of experimental groups B, C & D were prepared after mixing GIC with 5, 10 & 15 wt. % spider silk protein. All these groups were evaluated for their antibacterial activities by measuring the minimum Inhibition concentration as well as the formation of the Inhibition zone at respective concentrations by agar plate diffusion test. The collected data were recorded and subjected to a One-way ANOVA test for statistical significance. The statistical significance between the groups was determined using the post-hoc Tukey test. p is less than 0.05.
Results: The spider silk protein's minimal inhibitory concentration was 10 weight percent. Day 1, Day 7, Day 14, and Day 28 mean inhibition zone for control group A and experimental group B was 0.00mm. For experimental groups C and D, the mean inhibition zone at days 1, 7, 14, and 28 ranged from 0.6 mm to 2.7 mm. In comparison to control group A, experimental group B, and experimental group C, glass ionomer cement containing 15 weight percent SSP in experimental group D yields superior antibacterial activity results.
Conclusion: The Addition of spider silk protein in 10 & 15 wt. % increased the antibacterial activity against S. mutans.

Keywords: Antibacterial activity, Cervical caries, Glass Ionomer Cement, Recurrent Caries, Spider Silk Protein, Streptococcus Mutant.

INTRODUCTION

Materials used for direct restorations in the oral cavity need to be bioinert, meaning they shouldn't react with the surroundings. Materials including amalgam, composite resins, glass ionomer cement (GIC), and various cement varieties are frequently utilized. An early attempt was made to produce bioactive materials using alumino-silicate cement, or GIC, which has beneficial benefits against residual caries and releases fluoride ions. Over the past ten years, the idea of "smart" materials in dentistry has gained popularity. (1, 2).

The distinctive histological and structural features of non-carious cervical lesions present a special difficulty. The main etiological factors in their production are abfraction, abrasion, and erosion. Because these lesions are frequently subgingival, cleaning and treatment can be challenging. Commonly used materials for filling such cavities include GIC and composite resin, but their rough surfaces and subgingival margins can lead to biofilm accumulation, complicating oral hygiene (3-5).

Dental caries and other illnesses can result from disruptions in the micro-ecological balance of the dental plaque biofilm, which forms on all oral surfaces, including tooth restorative materials. Dental caries is mostly caused by Streptococcus mutans. In dentistry, a variety of restorative materials are employed, each with unique benefits and drawbacks, such as amalgam, composite resin, and GIC. (6-11).

GIC has garnered attention for its ability to release fluoride, modify physical properties, and exhibit antibacterial, adhesive, and esthetic properties. However, its fluoride release alone may not fully protect against bacterial growth. To enhance its antibacterial efficacy, various antibacterial agents and antibiotics have been added, albeit with potential compromises in physical properties (7, 12-14).
Spider silk protein has gained prominence in biomedical applications due to its biocompatibility, low immunogenicity, and antibacterial activity. Incorporating spider silk protein into GIC may enhance its antibacterial properties, potentially improving its efficacy in preventing or minimizing S. mutans incidence and enhancing its clinical application (15-17).
The literature and the study's explanation state that when fillers, extracts, or any antimicrobial agents are added, some properties get better while others get worse. Given that the potential goal of the study is to investigate the antibacterial effect of GIC in combination with SSP in preventing or limiting S. mutans, improving GIC's use in the clinic and lowering the occurrence of S. mutans.

METHODOLOGY

Sample scale: Eighty (80) experimental samples were manufactured and tested for antibacterial activity. (Table 1)

Table 1: Distribution of samples of several groups and sub-groups

Groups.	Sub Groups (aging) *	Silk (w/w %)	Number of specimens for subgroups	Total per Group.
Control A	A1	0%	5	20
	A2		5	
	A3		5	
	A4		5	
Experimental B	B1	5%	5	20
	B2		5	
	B3		5	
	B4		5	
Experimental C	C1	10%	5	20
	C2		5	
	C3		5	

	C4		**5**	
Experimental D	**D1**	**15%**	**5**	**20**
	D2		**5**	
	D3		**5**	
	D3		**5**	
Total Number of specimens		**80**		

*** Subgroup 1,2,3,4 indicates 1,7,14 & 28 days' period.**

Study Setting: The specimens used in this investigation were made specifically for antibacterial testing. conducted at Sardar Begum Dental College in Pakistan's Department of Science of Dental Materials.

Study duration: After receiving approval from the research proposal ethical committee, advance studies, and research board of Gandhara University Peshawar, Pakistan, this study was finished in six months**.**

Materials and Methods

The amounts of powder and liquid utilized in the production of the culture media used in Minimum Inhibitory Concentration (MIC) testing were carefully considered. To make the brain heart infusion broth, precisely 11.1 grams of powder were mixed with 300 milliliters of distilled water. To make sure this mixture was suitable for experimentation, it was autoclaved at 121°C and 1.5 bar of pressure. Similarly, different concentrations of spider silk protein (SSP) were carefully created by combining predetermined amounts of distilled water with 0.02 gm, 0.05 gm, 0.08 gm, 0.10 gm, and 0.15 gm of SSP.

Utilizing the generated brain heart infusion broth, S. mutans strains were cultured and standardized to a concentration of roughly 1.5 × 10^8 colony-forming units per milliliter (CFU/ml) for the MIC method. Then, various SSP solution concentrations were added to test tubes containing these standardized bacterial solutions. Solutions with 2%, 5%, 8%, 10%, and 15% SSP, for example, were assessed. The absence of turbidity in the culture media, which indicates inhibition of bacterial growth, was used to calculate the Minimal Inhibitory Concentration (MIC) during a 24-hour incubation period at 37°C.

Preparation of specimens involved careful consideration of powder-to-liquid ratios and precise measurements. Control group specimens were made with conventional glass ionomer cement (GIC), maintaining a ratio of 2.5 parts powder to 1 part liquid. Experimental groups included GIC mixed with SSP at different ratios, ranging from 9 gm of GIC powder and 1 gram of SSP to 8.5 gm of GIC powder and 1.5 gm of SSP. Using stainless steel molds, these mixes were formed into specimens and subjected to a number of quality control procedures, including sample selection based on predetermined standards such surface morphology and dimensional uniformity. After being exposed to bacterial strains for 24 hours, the specimens were examined for their antibacterial qualities against S. mutans using the agar diffusion method. This provided additional information about the specimens' efficacy.

Statistical analysis

The final results were recorded and calculated. Statistical analysis was conducted with SPSS 25, a software program. One-way analysis of variance and post hoc Tukey were utilized to determine the degree of significance between the groups under study for antibacterial activity. Procedures were carried out at a significance level of 0.05 or less.

Results

To evaluate the antibacterial activity, five specimens were generated for each group. The agar diffusion test method was used to determine the antibacterial activity, and the mean size of the halo zones, measured in millimeters (mm), was used to quantify the antibacterial activity. Among the

experimental and control groups, it was observed that group D exhibited the strongest inhibition against streptococcus mutans. (Table 4).
The Control Group A exhibited no ABA and presence of colonies of S. mutans around the specimens. The same was observed with the Experimental Group B . The mean size of IZ formed in group C & D are given in Table 3 & 4 respectively.

Table 3: Antibacterial activity of Experimental Group C

	Mean halo zones in mm			
Specimen No	**1 Day**	**7 Days**	**14 Days**	**28 Days**
1	0.8	1.6	1.6	0.6
2	1.5	2.5	1.6	2
3	0.5	2.3	2.1	1.5
4	1.5	2	2.6	0.8
5	1.1	2.3	1.8	2
Mean	2.52	2.14	1.94	1.38

Table : Antibacterial activity of Experimental Group D

	Mean halo zones in mm			
Specimen No	**1 Day**	**7 Days**	**14 Days**	**28 Days**
1	2.1	2.6	2.3	1.1
2	1.8	1.5	1.1	2.6
3	2	2.3	2.1	1.8
4	2.6	2.1	1.5	0.6
5	2.6	2.6	2.7	1.5
Mean	2.22	2.22	1.94	1.52

Using the One-way ANOVA test with p value < 0.05, it was determined that the antibacterial activities of the experimental groups C and D on days 1, 7, 14, and 28 were statistically significant when compared to the control group A. The outcomes showed that the glass ionomer cement's antibacterial qualities were conferred by the inclusion of spider silk protein.

Post hoc Tuckey Analysis

To determine the statistical significance between the groups, the post-hoc Tukey method was employed. There were six pairs formed between the groups A-B, A-C, A-D, B-C, B-D, and C-D. Within the Control group A and Experimental group B, no halo zone was developed on days 1, 7, 14, and 28. There was no evidence of antibacterial activity from these groups. When comparing experimental group C to control group A, there was a substantial difference in ABA between the two groups. When comparing experimental group D to control group A, there was a substantial difference in ABA between the two groups. When comparing experimental group D to control group A, there was a substantial difference in ABA between the two groups. In comparison to experimental group B, there was a substantial difference in the ABA of experimental groups C and B. When comparing Experimental Group D to Experimental Group B, there was a substantial difference in ABA between the two groups. In comparison to experimental group D, there was a substantial difference in the ABA of experimental groups C and D.

DISCUSSION

Non-carious cervical lesions, which manifest as defects in hard tissues at the cementoenamel junction due to carious lesions, are a common challenge in dental practice (28, 29). Among the various etiological factors contributing to these lesions, Streptococcus mutans stands out as a primary culprit. Notably, studies have demonstrated that biofilm growth is notably reduced in glass ionomer cements (GICs) containing quaternary ammonium salts compared to GIC control, suggesting a potential avenue for addressing this dental concern (7).
The release of fluoride from GIC is essential for improving remineralization and achieving cariostatic effects. Fluoride does, however, have a modest ability to inhibit bacterial development in GIC. The

quantity of fluoride released from GIC may not be enough to effectively stop bacterial development or stop bacterial deterioration of tooth tissues when GIC has been directly inserted, despite its cariostatic qualities. However, research showing that GIC is still among the more biocompatible materials for treating non-carious cervical lesions, as demonstrated by Bezerra et al.'s study, highlights its importance in clinical dentistry. (30).

The primary objective of our investigation was to determine whether adding spider silk protein (SSP) to GIC could provide it enough antibacterial qualities. According to our research, adding SSP to GIC powder provided antibacterial activity against S. mutans, a major cause of recurrent caries. These findings are in line with earlier studies, like the 2011 study by Silvia et al. that demonstrated the antibacterial activity of spider silk protein against Gram-positive and Gram-negative pathogens and linked this characteristic to particular antimicrobial domains in the protein structure. (31).

There is not ample study on SSP combined with GIC's antibacterial properties against S. mutans. We used the macro dilution method to find the minimal inhibitory concentration of SSP against S. mutans in order to close this gap. In addition to illuminating the possible antibacterial properties of SSP in GIC, our study's findings offer guidance for future investigations aimed at reducing SSP concentrations to reduce toxicity to the host tissues in the vicinity.. Interestingly, our findings revealed that increasing SSP concentration in GIC led to enhanced antibacterial activity against S. mutans, as corroborated by both agar plate diffusion tests and minimum inhibitory concentration results (32).

The agar plate diffusion test served as a crucial tool in evaluating the antibacterial activity of all specimens in our study. This method, widely used in antimicrobial research, has been employed by various studies to assess the efficacy of different agents against S. mutans and other bacteria. Notably, studies have demonstrated that spider silk protein, in its recombinant form, exhibits antibacterial properties against Streptococcus species, corroborating our findings (33).

Spider silk protein's antibacterial qualities seem to be mostly influenced by its structure, especially when it's coated in glycoproteins. Moreover, the combination of spider silk protein and antibacterial peptides increases their effectiveness, providing a possible substitute for traditional antibiotics. These results highlight spider silk protein's potential as a beneficial supplement to dental materials, with implications for preventing bacterial infections during a range of dental treatments. (34, 35).

CONCLUSION

Glass ionomer cement doped with 10 and 15 weight percent silk protein is antibacterial against Streptococcus mutans for 28 days.

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