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ASSESSMENT OF ANTIBACTERIAL EFFECT, SOLUBILITY, FLUORIDE ION RELEASE AND COMPRESSIVE STRENGTH OF GLASS IONOMER CONTAINING CHLORHEXIDINE VERSUS CONVENTIONAL GLASS IONOMER RESTORATIVE MATERIAL (IN-VITRO STUDY)

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ABSTRACT

Aim: The current study's aim was to compare the antibacterial effect of chlorhexidine (CHX) modified glass ionomer cement (GIC) using Consepsis (2% CHX gluconate solution) versus conventional GIC against *Streptococcus mutans* and *Lactobacillus acidophilus*. In addition, solubility, fluoride ion release and compressive strength of the two tested groups were compared.

Materials and methods: Antibacterial efficiency of both material groups was evaluated using agar diffusion test. Total of 200 samples were prepared for this test where the inhibition zones were measured in mm and tested at various time intervals (1, 7, 14, 21, and 28 days). Ten-disc shaped samples were used to measure solubility ($\mu g/mm^3$) by detecting their weight change prior to and following a 28-day submersion in distilled water. Fluoride ion release was measured using ten-disc shaped samples and its amount determined in mg F/L after 28 days. A universal testing machine was used to measure the compressive strength (MPa) of ten-cylinder shaped samples for each material.

Results: Results of antibacterial test showed a statistically significant increase in inhibition zones(mm) of the intervention group compared to the control group against *Streptococcus mutans* and *Lactobacillus acidophilus* in the all-time intervals of the test (p-value ≤ 0.05). Results of solubility ($\mu g/mm^3$), fluoride ion release (mg F/L) and compressive strength (MPa) indicated that there was no significant difference between the CHX modified GIC and the conventional group.

Conclusion: The addition of Consepsis (2% chlorhexidine gluconate solution) to the conventional Fuji IX glass-ionomer cement promoted the antibacterial effect against *Streptococcus mutans* and *Lactobacillus acidophilus* with no deteriorating effect on, solubility, fluoride ion release and compressive strength.

KEYWORDS: Chlorhexidine, glass ionomer cement, Consepsis, of Oral and dental medic.

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INTRODUCTION

One of the most severe mouth diseases is caries of the teeth. As a result, several efforts have been performed to treat this disease. Dental cavities affect between 60 and 90 percent of adults and school-age children globally. Bacteria and food consumption are of the main factors that can lead to dental caries due to carbohydrate fermentation producing high number of acids [1]. Many studies [1-3] had indicated that maintained biofilm development had a major influence on caries etiology. Furthermore, cariogenic bacteria cause demineralization of dental hard tissues and leads to formation of biofilms and subsequently to formation of dental decay.

It was shown that the primary causes of secondary caries at the interface between cavity preparations and dental restorations were acid-producing bacteria, specifically *Streptococcus mutans* and *Lactobacillus acidophilus*. Therefore, materials that restrict bacterial growth, surface colonization, and minimize acid generation by microorganisms are preferable [2, 3].

Among the dental materials most frequently utilized in standard dental procedures are glass ionomer cements (GICs) [4]. In the early 1970s, Wilson and Kent invented the usage of glass ionomer cements (GICs). GICs have special properties such as biocompatibility, long-term fluoride release imparting anti-cariogenicity, flexibility simulating dentin, and adhesion to the tooth structure.

GICs acquire popularity as dental material for these mentioned reasons. In pediatric dentistry, they are widely used as restorative materials. These include fissure sealants, lining and base materials, and materials for Atraumatic Restorative Treatment (ART) [4].

Although GICs have many benefits, they also have significant disadvantages, such as secondary caries, which is the most frequent reason for restoration failure, and insufficient fluoride release from GICs to prevent bacterial growth. This has restricted their widespread usage in dentistry as restorative material [6].

Antibacterial materials have recently been added to GICs in an attempt to improve their antibacterial properties. It was thought that adding these possible materials would improve GIC's characteristics and increase its uses in clinical dentistry. Recent developments include the introduction of antibiotics such as metronidazole, minocycline, and ciprofloxacin. In addition, chloroxylenol, boric acid, thymol, cetrimide (CT), triclosan, benzalkonium chloride and cetylpyridinium chloride were included into GICs to enhance their antibacterial qualities. The addition of antimicrobial agents to GICs can have a negative impact on the other physical and/or adhesive qualities. Antibacterial effect was primarily determined by the amount and nature of the antimicrobial agent utilized, as well as the rate at which it was released from the specimen's surface layer [4]. When antimicrobial agents are added to GICs, the physical and mechanical characteristics of the restorative materials are altered [7-9].

Chlorhexidine is regarded as one of the most efficient and safe antimicrobial products used to manage oral microbes. It works widely against gram-negative, facultative anaerobic, aerobic, and gram-positive bacteria, including Streptococci mutans, as well as yeasts and fungi [7].

In previous studies [7-9], researchers added chlorhexidine in various concentrations (0.5, 1.5, and 2.5% by weight) and they found that, the 2.5% concentration increased the antimicrobial activity of GICs; but adversely affected other properties such as mechanical properties.

Accordingly, the aim of this research was to add Consepsis (2% chlorhexidine digluconate solution, Ultradent, USA) to conventional glass-ionomer restorative material to improve its antibacterial action and evaluate its effect on the solubility, fluoride ion release and compressive strength.

The null hypothesis stated that there would be no difference between chlorhexidine (CHX) modified GIC versus conventional GIC regarding their antibacterial activity, solubility, fluoride ion release and compressive strength.

MATERIALS AND METHODS

Samples preparation

For the intervention group (G2), according to the pilot study, conducted to determine the proper P/L according to manipulation and antibacterial effect, drops of chlorhexidine antibacterial solution (Consepsis®, Ultradent, USA: 2% chlorhexidine gluconate solution) was added to one drop of the liquid of conventional glass ionomer cement onto a cool, dry glass slab, then incorporated and mixed thoroughly with the powder using a stiff plastic spatula for the intervention group (G2).Specimens of the conventional GIC (G1) were proportioned in accordance with the manufacturer's instructions, as single scooping of powder to single drop of liquid equivalent to 3.6g/1.0g by weight and mixed thoroughly as mentioned before.

A total number of 200 GICs samples had been prepared in this study. The samples were separated into two primary groups based on material type, and each group was further divided into four subgroups based on test type, with the agar diffusion test being done at different time intervals (1, 7, 14, 21, and 28 days). as shown in **figure (1).**

Antibacterial activity

Where is sample size calculation for each test and their references as written in thesis. Should be written before antibacterial test.

The antibacterial activity of the two materials under investigation was assessed using the agar diffusion test against *Lactobacillus acidophilus* (ATCC® 314TM) and *Streptococcus mutans* (ATCC® 25175TM). Each group's seventy-disc-shaped samples were further divided into two subgroups based on the type of bacteria, with thirty-five samples each. Five subgroups (n=7) were created from each group in order to test them at various intervals of time (1, 7, 14, 21, and 28 days)[8].

Seventy agar plates (7 plates / sub-group) were prepared with Brain Heart Infusion (BHI) agar for *Streptococcus mutans* and Lactobacillus MRS Agar for *Lactobacillus acidophilus*, as shown in **figure (2)**. Each plate contained one disc sample from each material. 20 ml of agar was poured and left to solidify. Then, 0.1 ml of microbial suspension was measured using an automatic micropipette and placed on each agar plate. The bacterial suspension was spread in two directions to give homogenous growth by using a sterile cotton swab[9], as shown in **figure (3)**.

After 24 hours of incubation for the *Streptococcus mutans* group and 48 hours of incubation for the *Lactobacillus acidophilus* group, the results were scored. However, to assess anti - microbial activity for longer periods the test was also conducted after 7, 14, 21 and 28 days of incubation as mentioned previously, and the results were scored[8, 10].

Solubility

Ten disc-shaped samples were used to determine solubility. The samples were weighed using the precision weighing scale (Adam Equipment, UK) to determine the initial mass (M1). Each group's samples were placed in tightly sealed plastic tubes and submerged in ten milliliters of distilled water. The plastic tubes were stored on incubator (Helena-labs, USA) at 37±2˚C for 28 days. After taking the samples out of the distilled water, the excess water was wiped off with filter paper and dried at 37˚C for another 24 hours in a desiccator containing silica gel. The samples were weighed to calculate their final dry mass (M2)[11-14].

The solubility was estimated using the equation below:

$$Wsol = \frac{M1 - M2}{V}$$

Where:

Wsol: solubility of test material ($\mu g/mm^3$).

M1: mass of the samples before immersion in water (μg).

M2: the final dry mass of the samples (μg).

V: volume of the samples (mm^3).

Fluoride ion release

For the measurement of the fluoride ion release ten-disc shaped samples were used. The set samples after being removed from the molds were immersed in 10 ml distilled water in plastic test tubes, samples were shaken and then immediately incubated at 37°C for 28 days[43, 62]. A digital ion analyzer was connected to a fluoride-specific ion electrode to measure the amount of fluoride ions released from the prepared samples in distilled water (DM-20P, Digimed, Brazil). Each sample was measured by adding 0.5 ml of the premade total ionic strength adjustment buffer solution TISAB II to 5 ml of distilled water, which served as the storage medium. The fluoride concentration was measured in ppm (mg F/L)[15-18].

Compressive strength

Ten-cylinder shaped sample/group were used for compressive strength test with dimensions of the flat ends of each sample were placed between the plates of the universal testing apparatus (Instron type 3345, England). The compressive force (N) was applied to the samples along their long axis at a crosshead speed of 1mm/minute [19-21]. The following formula was used to determine the compressive strength in MPa:

$Cs = (4P)/ (\pi d^2)$.

Where P was the greatest force applied in Newtons, d was the average measured diameter of the sample in mm and π was the numerical constant of 3.14.

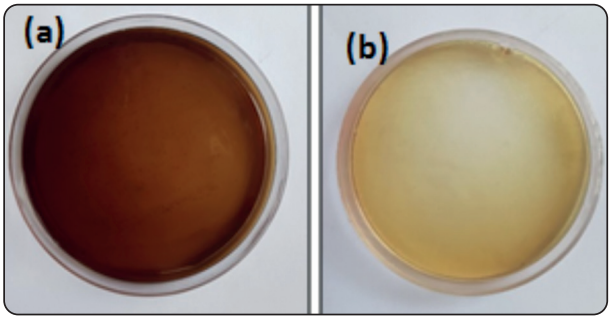

Fig. (1): (a) Brain Heart Infusion (BHI) agar used for Streptococcus mutans. (b) Lactobacillus MRS Agar used for *Lactobacillus acidophilus*.

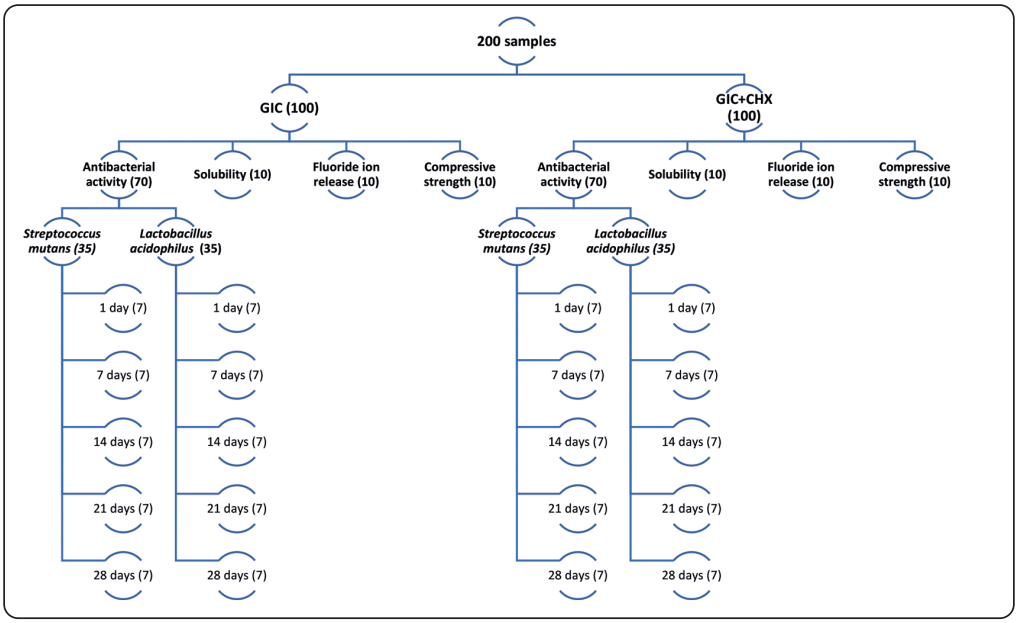

Fig. (1): Samples grouping of the study.

RESULTS

Antibacterial activity against *Streptococcus mutans*:

The highest inhibition zone mean value against *Streptococcus mutans* was recorded after 14 days for the intervention group (18.2 mm) while the lowest inhibition zone mean value was recorded after 1 day and 28 days for the control group (10 mm). The inhibition zones of the intervention group against *Streptococcus mutans* were significantly higher than those of the control group in all tested time intervals (p-value ≤ 0.05) **table (1)** and as shown in **figure (4).**

TABLE (1) Mean values and standard deviation of inhibition zone (mm) of the tested groups against *Streptococcus mutans*

Group / Time intervals	G1 (GIC) (mm)	G2 (GIC + CHX) (mm)	P value
Day 1	10±0.7	14±1	0.0001*
Day 7	12 ±0.7	15.8±1	0.0001*
Day 14	12.2±0.4	18.2±1.1	0.0001*
Day 21	11.2±0.8	14.6±0.9	0.0001*
Day 28	10±0.7	13.2±0.8	0.0001*

: significant (p≤ 0.05).

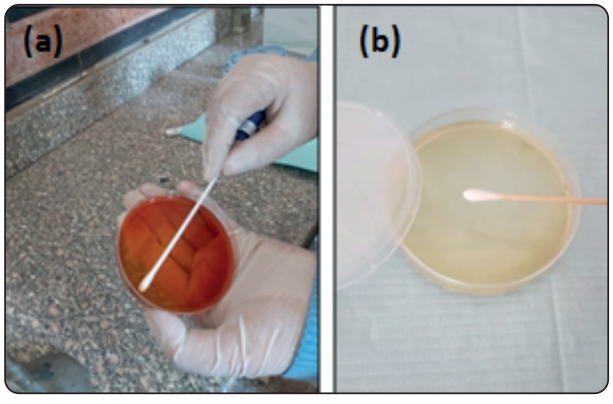

Fig. (2) The surface of each agar plate swabbed by a sterile swab. (a) *Streptococcus mutans* and (b) *Lactobacillus acidophilus*

Antibacterial activity against *Lactobacillus acidophilus*:

The highest inhibition zone mean value against *Lactobacillus acidophilus* was recorded after 14 days for the intervention group (18.2 mm) while the lowest inhibition zone mean value was recorded after 1 day and 28 days for the control group (10 mm). The inhibition zones of the intervention group against *Lactobacillus acidophilus* were significantly higher than those of the control group in all tested time intervals (p-value ≤ 0.05) **table (2)** and as shown in **figure (5).**

TABLE (2) Mean values and standard deviation of inhibition zone (mm) of the tested groups against *Lactobacillus acidophilus*.

Group / Time intervals	G1 (GIC) (mm)	G2 (GIC + CHX) (mm)	P value
Day 1	10±0.6	11.4±1.2	0.001*
Day 7	11.2±0.7	14.6±0.8	0.0001*
Day 14	12.2±0.4	18.2±1	0.0001*
Day 21	12.6±0.8	14.8±0.7	0.0001*
Day 28	10±0.6	14±0.9	0.0001*

: significant (p≤ 0.05).

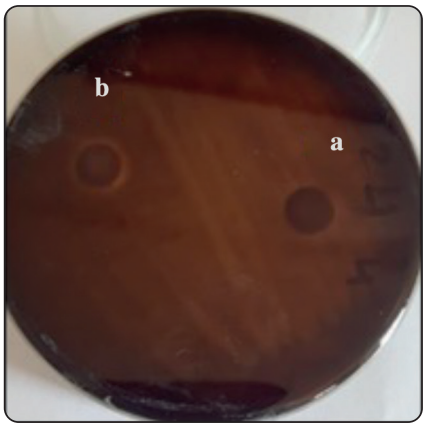

Fig. (3) Inhibition zone of one selected sample of the tested groups against *Streptococcus mutans*. (a) Inhibition zone of GIC. (b) Inhibition zone of GIC with CHX. (Day 1).

Solubility, fluoride release and compressive strength: There was no significant difference in the solubility(μg/mm^3), fluoride ion release (mg F/L) and compressive strength (MPa) values between the intervention group and the control groups (p-value >0.05) as shown in **tables 3,4 and 5** respectively.

Table (3): Mean values and standard deviation of solubility for control and intervention groups (μg/mm^3)

Group / Test	G1 (GIC) μg/mm^3	G2(GIC + CHX) μg/mm^3	P value
Solubility	0.06± 0.01	0.07± 0.01	0.16

Table (4): Mean values and standard deviation of fluoride ion release for control and intervention group (mg F/L)

Group / Test	G1 (GIC) mg F/L	G2(GIC + CHX) mg F/L	P value
Fluoride release	7.5 ± 0.9	8.2 ± 2.4	0.6

Table (5): Mean values and standard deviation of compressive strength for control and intervention groups (MPa)

Group / Test	G1 (GIC) MPa	G2 (GIC + CHX) MPa	P value
Compressive strength	92.2 ± 27.9	71.6 ± 17.3	0.2

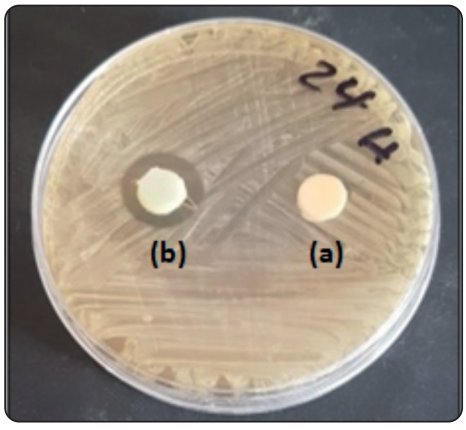

Fig. (4): Inhibition zone of one selected sample of the tested groups against *Lactobacillus acidophilus*. (a) Inhibition zone of GIC. (b) Inhibition zone of GIC with CHX (Day 1).

DISCUSSION

In the current study minimum concentration of CHX gluconate in the form of Consepsis was added to the conventional GIC as ***Takahashi et al* 2006** [9]found that the antibacterial efficacy of GIC was not influenced by the CHX concentration. The minimum concentration of CHX applied to GIC can avoid occurrence of adverse effects on other properties of the cement.

The agar diffusion test was used in this study to assess the antibacterial activity of the control (conventional GIC) and the intervention (CHX modified GIC) against *Lactobacillus acidophilus* and *Streptococcus mutans*. Because *Streptococcus mutans* is the main bacteria that cause caries, and *Lactobacillus acidophilus* is the main bacteria associated to the progression of caries, these micro - organisms were selected [23]. The first time interval was one day for *Streptococcus mutans* group and two days for *Lactobacillus acidophilus* group, which represented the time required for the microorganisms to grow according to the conducted pilot study and previous literature[15, 30].

The agar diffusion test used in our investigation showed that Fuji IX GIC (conventional) exhibited antibacterial activity against *Lactobacillus acidophilus* and *Streptococcus mutans*. These results consistent with those of ***Shashibhushan et al.* 2008**[24], who showed that GICs released fluoride ions into an aqueous medium, which may inhibit bacterial growth, and thus inhibited the growth of *Lactobacillus acidophilus* and *Streptococcus mutans*. On the contrary, other studies[25, 26] revealed that when it came to *Streptococcus mutans* and *Lactobacillus acidophilus*, Fuji IX GIC showed no antibacterial activity. This could have been caused by a number of factors, including how the material was prepared, its P/L ratio, how long it was manipulated, temperature, sample shape, surface protection, and how the medium was stored or dissolved.

The antibacterial activity results of CHX modified GIC was in agreement with ***Sanders et al in* 2002**[27], ***Takahashi et al in* 2006**[9], ***Türküün et al* 2008**[8], ***Marti et al* 2014**[28], ***Yadiki et al 2016***[29]**and *Kurt et al. 2021***[30] as the bacterial growth of *Streptococcus mutans* and *Lactobacillus acidophilus* was inhibited when chlorhexidine gluconate solution was added to glass ionomer liquid; however, the effective antibacterial effects gradually decreased as a result of a decrease in the amount of CHX that was available, emphasizing the role of CHX in enhancing the antibacterial potential [27].

Since the oral cavity is humid, the restorative materials network can take in water and chemicals from the environment and then release those components back into the environment. The addition of antibacterial agents may affect moisture sensitivity and contribute to hydrolytic degradation of GIC; thus, it was necessary to assess whether adding CHX would negatively impact GIC's solubility[31].

In the current investigation, there was no significant difference in the solubility test results between the intervention group and the control group. This may imply that CHX solution addition did not adversely affect dissolution tendency of GIC which could be considered advantageous. These findings were similar to, ***Yan. H. et al,* 2017**[31] regarding low concentration of CHX (1-5%). However, addition of high concentration as 10% CHX increased solubility rate (1.3%). They explained that increasing the concentration of CHX increased the rate of water solubility because CHX has a nano-porous structure that gives multiple channels for water to penetrate into GIC.

In contrast to our findings, negative solubility values were reported by ***Toledano et al. in* 2006**[32], ***Keyf et al. in* 2007**[33], ***Sinthawornkul at el. in* 2017**[34], and ***Singer et al,* 2020**[35]. This could be explained by the partial dehydration of these specimens at the beginning of the test which might be due to the initial storage conditions before complete setting; this did not necessarily imply that no solubility took place, although it may give some indication of it[35].

The study found no statistically significant variation in fluoride ion release between the intervention group and the control group. In accordance with ***Tüzüner et al,* 2011**[37]who demonstrated similar fluoride release pattern in both GIC (additive-free) or CHX modified GIC.

In disagreement to the findings of the present investigation, ***Da Silva et al,* 2019**[38] observed that the fluoride release from the glass ionomer cement group mixed with 1.25% CHX was nearly double that of the conventional GIC group. However, a problem evolved as it was found that CHX gluconate has a great affinity for hydroxyapatite. The structure of the CHX molecule is similar to that of an amino acid, and its affinity for calcium sites is increased by the presence of negatively charged centers. Hence, the amount of fluoride adsorbed to hydroxyapatite is decreased when CHX is present, most likely as a result of F and CHX adsorbing to the same binding sites on the hydroxyapatite in competition.

Antibacterial drugs have been shown in several studies[8, 9]to alter the mechanical characteristics of glass ionomer cements. The compressive strength of the control and intervention groups did not differ significantly in the current investigation.

These findings were consistent with ***Takahashi et al in* 2006**[9], ***Ahluwalia et al,* 2012**[39], ***Hu et al in* 2013**[40]and ***Duque et al in* 2017**[36]. This might be because GIC contained small amounts of chlorhexidine (ranging from 1%-2.5%), which did not prevent the glass ionomer network from forming.

In contrast, ***Jedrychowski, Caputo and Kerper in* 1983**[41]. ***Marti et al,* 2014**[28] found that the addition of CHX at concentrations greater than 5% caused the glass ionomer cement to deteriorate, and attributed the loss in compressive strength with higher concentrations of CHX diacetate or digluconate to an increase in porosity, which most probably reduced strength.

According to ***Sanders et al in* 2002**[27] and ***Türkün et al* 2008**[8] The reason for the decrease in the physical properties of GICs altered by CHX digluconate liquid might be that CHX dissolves more quickly in the external environment when it is in the form of CHX digluconate liquid than when it is in the form of CHX powder or diacetate. ***Yadiki et al 2016***[29], reported that another reason for the decrease in mechanical properties was that high concentrations of chlorhexidine would weaken and compromise the physical properties of the antibacterial glass ionomer cements by interfering with the cement's setting process. Thus, the amount of chlorhexidine should be kept as low as possible.

As can be detected from the current study, the null hypothesis was rejected as regards the antibacterial activity. However, failure to reject the null hypothesis occurred regarding solubility, fluoride release and compressive strength.

CONCLUSION

Based on the limitations of the current study, it is possible to conclude that:

1) The addition of 2% chlorhexidine gluconate (Consepsis) to the conventional Fuji IX glass-ionomer cement promoted its antibacterial effect against *Streptococcus mutans* and *Lactobacillus acidophilus*.
2) The addition of Consepsis (solution of 2% chlorhexidine gluconate) to the conventional Fuji IX glass-ionomer cement did not exert deteriorating effect on the compressive strength, solubility and ion fluoride release rate.

Conflict of Interest:

The authors declare no conflict of interest.

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Ethics:

This study protocol was approved by the ethical committee of the faculty of dentistry-Cairo University.

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