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Using bioactive glass alone or combined with calcium hydroxide modifies dentin biological and mechanical properties: An in-vitro study.

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Abstract
Introduction: Calcium hydroxide ($Ca(OH)_2$) is the most commonly used medication in endodontic treatments. This study aimed to examine the impact of bioactive glass (BG), both with and without $Ca(OH)_2$, on dentin biological and mechanical properties.
Materials and Methods: Standardized holes were created on 84 dentin slices to replicate root canals. The samples were divided into four groups based on the medicament placed in the root canals: $Ca(OH)_2$, $Ca(OH)_2$+ 7% BG, BG, and a control group. Scanning electron microscopy (SEM) was used to analyze the dentin surfaces exposed to the medicaments. At the same time, energy dispersive X-ray (EDX) analysis was conducted to assess apatite formation at 1, 7, and 14 days. X-ray diffraction (XRD) measurements were taken to examine the formation of mineral phases. Additionally, microhardness and pH levels were measured. Statistical analysis was performed using the Kruskal-Wallis and Wilcoxon signed-rank tests ($P < 0.05$).
Results: The $Ca(OH)_2$ and control groups exhibited the highest levels of calcium (Ca) and phosphorus (P) mineral content on the 14th day, while the lowest Ca and P content was observed in the Ca(OH)2 + BG group ($P < 0.05$). A gradual reduction in crystal formation was noted in the Ca(OH)2 + BG group from day 1 to day 14. The highest microhardness values were also recorded in the Ca(OH)2 + BG group ($P < 0.05$). The $Ca(OH)_2$ + BG group also demonstrated the highest pH readings ($P = 0.001$).
Conclusion: $Ca(OH)_2$+ BG could serve as a potential alternative intracanal medicament. Additional studies are needed to explore the clinical implications of this combination.

INTRODUCTION:

Medicaments are usually used in endodontics to eliminate living bacteria that cannot be removed from the root The canal system, as a result of chemo-mechanical preparation, aims to reduce periapical inflammation and pain, prevent inflammatory root resorption, and act as a barrier within the root canal system.[1,2] Calcium hydroxide ($Ca(OH)_2$) is the most widely used medicament in endodontics due to its antibacterial, therapeutic, biocompatible, and regenerative properties.[2,3] It is a highly alkaline substance with a pH of around 12.5, breaking down into calcium and hydroxy ions in an aqueous solution.[4] Its low solubility in water encourages periapical hard tissue formation around infected root canals[5] and contributes to periapical tissue healing, even after trauma.[6] $Ca(OH)_2$ is often considered the "gold standard" for endodontic medicaments; however, it may not effectively eliminate some facultative bacteria and fungi in root canal[7] systems. Moreover, prolonged use of $Ca(OH)_2$ as an intracanal dressing can negatively

impact the mechanical properties of radicular dentin, potentially leading to root fractures. As a result, alternative materials such as bioactive glasses (BG)[9] have been investigated to address the limitations of $Ca(OH)_2$in endodontic treatments.
Bioactive glass (BG) is a valuable material that supports hard tissue healing and has gained particular attention in endodontic treatments due to its regenerative and antimicrobial properties. Its chemical composition closely resembles that of human bone and dentin.[10,11] In vivo studies have shown that BG is associated with favorable cellular and inflammatory responses in the pulp when used in direct pulp capping procedures, often alongside mineral trioxide aggregate.[12] The combination of BG with various materials, such as zinc oxide nanoparticles, toothpaste, and nano-tricalcium silicate, has also been explored in the literature.[13-15] Previous research has examined the apatite formation and ion release properties of a BG and $Ca(OH)_2$ mixture, though it did not include dentin samples.[16] This study aims to investigate whether BG alters the properties of radicular dentin, such as microhardness and mineral structure, when used alone or in combination with $Ca(OH)_2$, and whether it could serve as an alternative to $Ca(OH)_2$. Specifically, the study evaluates the effects of BG, with and without $Ca(OH)_2$, on the biological and mechanical properties of radicular dentin, as well as the pH changes associated with these treatments.

MATERIALS AND METHODS

The Faculty of Dentistry Ethical Committee approved the study protocol at Rama Dental College, Hospital and Research Centre, Kanpur.

The production of **45S5 bioactive glass (BG)** samples was carried out using the classical melting method in a high-temperature muffle furnace (Protherm, Ankara, Turkey). The resulting BG structure had the following composition by weight: 45% SiO2, 24.5% CaO, 24.5% Na2O, and 6% P2O5. The mixture was first placed in a platinum crucible and melted at 1400°C and 1450°C for 1 and 2 hours, respectively. After melting, the mixture was poured onto an iron bench in drop form and annealed at 550°C for 24 hours. The resulting 45S5 BG samples were then mechanically crushed and ground using an agate mortar to achieve a particle size of less than 100 μm.

Preparation of samples

Based on power analysis (using an alpha level of 0.05 and a power of 0.80), a minimum of 20 samples, with five samples per group, was required. Since some samples could not be reused 84 upper premolar teeth were selected, all extracted for orthodontic or periodontal reasons and free of caries, cracks, fractures, or restorations. Radicular dentin sections, 4 mm thick, were obtained from the coronal portion of the teeth using a device (Mecatome T180, Presi, Grenoble, France) and cutting discs (DIMOS Ø100 19-100), under water cooling. Standardized 1.2-mm diameter holes, simulating root canals, were created in the dentin discs using a fissure-shaped diamond bur (841H012, Meisinger, Hager and Meisinger GmbH, Heisinger, Germany). The dentin discs were then polished using a polishing machine (Minitech 233, Presi, France) to remove debris. The samples were stored in stimulated body fluid until the experiment. The samples were then randomly divided into four groups, with 21 in each group.

Group 1: The $Ca(OH)_2$ medicament (Kalsin, Turkey) was prepared by mixing $Ca(OH)_2$ powder and distilled water in a 1:1 ratio, following the manufacturer's instructions. The prepared medicament was then placed into the holes on the dentin slices using K-type hand files (Shenzhen Denco Medical Co., Ltd., China). Condensation was achieved with a hand instrument, and any excess material was removed using a handpiece.

Group 2: A mixture of $Ca(OH)_{2\ powder}$ and 7% bioactive glass (BG) was prepared by mixing with distilled water in a 1:1 ratio. The mixture was then placed into the holes, simulating the root canal. Condensation was performed using the same hand instrument, and excess material was removed using a handpiece.

Group 3: Bioactive glass (BG) was mixed with distilled water in a 1:1 ratio and placed into the holes.

Group 4: No medicament was placed.

The discs were initially kept at room temperature to allow the materials to harden. Afterward, they were transferred to separate Eppendorf tubes and placed in an oven. All samples were stored at 37°C in 100% humidity until the day of the experiment.

Scanning electron microscopy-energy dispersive X-ray analysis

Scanning electron microscopy (SEM) (EVO LS 10, Zeiss, Carl Zeiss, Germany) was used to assess the surface morphologies of the groups and to observe apatite formation following artificial body fluid studies. Energy dispersive X-ray (EDS; EDAX, Gatan Inc., CA, USA) analysis was conducted for semi-quantitative elemental examination of the sample surfaces in contact with the medicaments. To ensure clear morphological imaging, the samples were dried and then coated with a 5 nm thick layer of gold under 15 kV power using the SE detector. The samples were examined at various magnifications (×250, ×1000, ×3000, and ×10,000).

Secondary electron and backscattering methods were applied to evaluate the surface images. EDS analyses were conducted on the dentin near the interface, with samples taken from three points at four different regions. The surfaces of the dried samples were coated with a thin gold-palladium layer in an airless environment for elemental analysis. Rectangular areas on the images obtained at magnifications of ×250, ×1000, ×3000, and ×10,000 were marked using a computer, and the mineral contents were examined. The atomic and weight percentages (%at and wt%) of elements such as Ca, Cl, K, Zr, Na, Mg, Si, P, O, and Na ions were calculated from the analysis.

X-ray diffraction diffractometers

X-ray diffraction (XRD) analysis was meticulously conducted on days 7, 14, and 21 to gain valuable insights into the material properties. Employing a precise scanning method at a rate of 2°/min, we covered a crucial angle range of $2\theta° = 3–50$. The X-rays were expertly generated at 40 mA and 45 kV through a CuKα anode tube, maintaining optimal conditions at

25°C (D8 Advance Bruker, Germany). A comprehensive examination of seven samples was conducted: two from each of the initial three groups and one from the fourth group. We carefully analyzed the crystal phase peaks from the mineral structures within the XRD patterns, revealing critical information about the materials under study.

pH Measurement

The pH changes induced by the sample groups incubated in Dulbecco's phosphate-buffered saline (PBS) medium for specific periods (1, 7, and 14 days) were measured using a pH meter (WTW/pH 3310). A total of 13 samples from each group were used for this analysis.

Microhardness analysis

A Vickers microhardness test was conducted on five dentin sections from each group using a Hardway-MHVS 1000 AD indentation device (China) on the 1st, 7th, and 14th days after applying the intracanal medicament. The indentations were made with a Vickers diamond indenter positioned 100 μm from the surface where the medicament interacts with the dentin, applying a force of 100 g for 15 seconds.

Data collection and statistical analysis

The Shapiro–Wilk test was performed to assess the normality of the data distribution before analysis. The results indicated that the distribution was not normal. Consequently, all data were analyzed using the Kruskal–Wallis test. For comparisons of time-based measurements, the Wilcoxon signed-rank test was applied ($P < 0.05$).

RESULTS

Scanning electron microscopy-energy dispersive X-ray

The elemental content and percentages obtained from the EDX analysis of the test groups are presented in Figure 1. The analysis revealed that the mineral deposits formed in all groups contained Ca, Cl, K, Zr, Na, Mg, Si, P, O, and Na ions. After 14 days, the highest oxygen content (33.63 ± 17.01) was observed in the $Ca(OH)_2$ group, while the lowest value (24.33 ± 11.26) was recorded in the BG group ($P = 0.001$). By day 14, the highest calcium content was found in both the BG group (28.49 ± 3.28) and control group (28.49 ± 5.65), while the lowest level was seen in the $Ca(OH)_2$+BG group ($P = 0.001$). The BG group consistently exhibited the highest amount of silicon ions across all time points ($P < 0.001$). On the 14th day, the BG group (3.24 ± 2.42) and the control group (3.59 ± 2.73) had the highest phosphorus levels ($P = 0.025$). A SEM image of the $Ca(OH)_2$+BG group is shown in Figure 2.

X-ray diffraction analysis

The results of the XRD analysis for selected samples from each group on the 1st, 7th, and 14th days are displayed in Figure 3. The diffraction peaks characteristic of crystals, expected to consist of Ca-Si-P atoms, were observed at 26°, 32°, and 46°. It was found that the

$Ca(OH)_2$+BG group exhibited less crystal formation on the 14th day. A gradual reduction in crystal formation was observed in the $Ca(OH)_2$+BG group from day 1 to day 14, while the BG group showed a gradual increase in crystal formation over the same period.

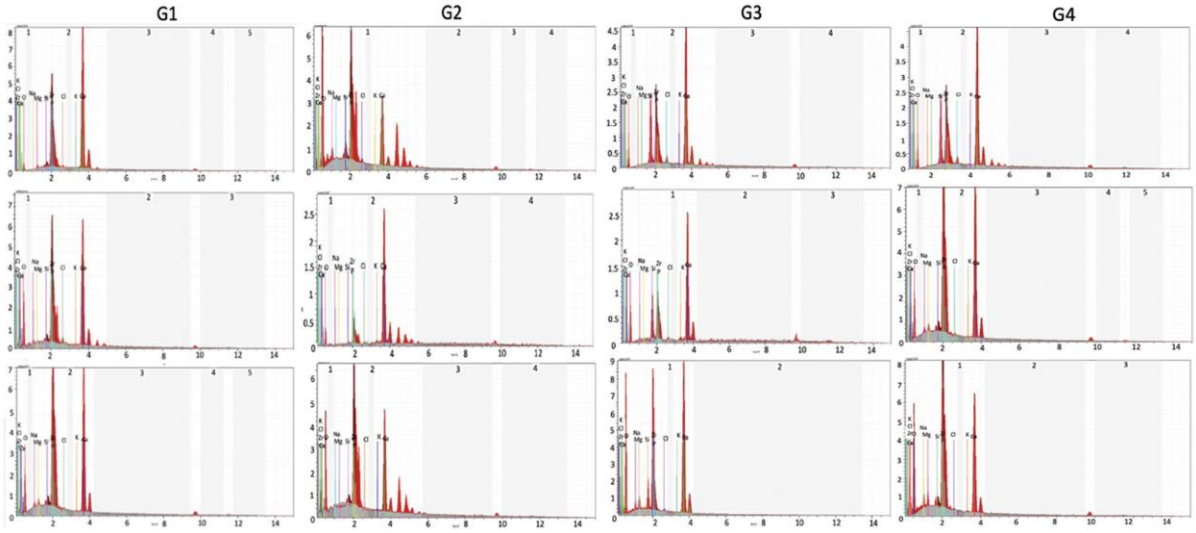


Table 1: Statistical analysis results of pH measurements of all groups

pH	$Ca(OH)_2$	$Ca(OH)_2$+BG	BG	Control	P
1st day	7.12 ± 0.05^{a1}	7.20 ± 0.16^{b1}	6.94 ± 0.03^{c1}	6.96 ± 0.04^{c1}	0.001
7th day	7.31 ± 0.16^{a2}	8.21 ± 1.01^{b2}	7.03 ± 0.06^{c1}	7.05 ± 0.06^{c1}	0.001
14th day	7.63 ± 0.41^{a3}	8.40 ± 1.21^{b3}	7.15 ± 0.12^{c1}	7.08 ± 0.08^{c1}	0.001
P	0.012	0.001	0.752	0.860	

Different letters in the same row and different numbers in the same column show statistically different groups ($P<0.05$). BG: Bioactive glass, Ca (OH)2: Calcium hydroxide

pH analysis

Table 1 presents the pH analysis results for the 1st, 7th, and 14th days. The $Ca(OH)_2$+ BG group exhibited the highest pH values on all three days (P = 0.001).

Microhardness analysis

Dentin microhardness measurements are provided in Table 2. A statistically significant difference in microhardness was observed between the four groups on the 14th day (P < 0.05). The highest microhardness was recorded in the $Ca(OH)_2$+ BG samples, while the lowest was found in the control group (P = 0.001).

The major steps involved in the study design and its outcomes have been highlighted in the PRILE 2021 flowchart, as depicted in Figure 4.

DISCUSSION

Yassen and Platt reported that intracanal medicaments can change the chemical structure of dentin.[17]. The strong alkalinity of $Ca(OH)_2$ can potentially lead to the collapse of the dentin structure by denaturing carboxylate and phosphate groups,[18] despite its broad antimicrobial,

therapeutic, biocompatible, and regenerative effects.[2,3] This has prompted investigations into new antimicrobial agents as alternatives to $Ca(OH)_2$ [19]Bioactive glass (BG), composed of phosphate and silica, is biocompatible and can form chemical bonds with bone tissue.[20] It is widely used as a mineralizing and desensitizing agent in caries prevention and sensitivity treatments due to the apatite formation from the release of Si, Ca, and PO4 ions. These minerals, including Ca, P, and F, are crucial for inhibiting demineralization, stimulating remineralization, and forming the core structure of acid-resistant fluorapatite compounds.

In the present study, SEM/EDX results confirmed that BG contains K, Zr, Ca, O, Cl, Na, Mg, Si, and P ions. The BG group showed the highest amounts of Ca and Si ions. Si was found to be the most abundant in the BG group across all periods ($P < 0.001$). On the 14th day, the highest amount of oxygen was observed in the $Ca(OH)_2$ group, whereas the lowest was in the BG group ($P = 0.001$). The high presence of Ca, O, and Si in BG suggests that these materials support biomineralization or bioactivity. Any alteration in the Ca/P ratio can affect the balance of organic and inorganic components, which in turn influences the permeability and solubility of dentin.[21] In this study, after 14 days, the groups 1st and 4th exhibited the highest Ca and P mineral content, whereas the $Ca(OH)_2$+ BG group showed the lowest Ca and P content.

Table 2: Comparison of all groups based on microhardness measurements

MICROHARDNESS	$Ca(OH)_2$	$Ca(OH)_2$+BG	BG	CONTROL	P
1st day	77.72 ± 3.54^{a1}	76.20 ± 9.07^{a1}	66.38 ± 12.3^{b1}	53.53 ± 2.32^{c1}	0.001
7th day	72.70 ± 8.66^{a2}	69.38 ± 10.0^{a2}	53.56 ± 2.28^{b2}	55.05 ± 3.32^{b1}	0.001
14th day	66.50 ± 1.90^{a3}	72.50 ± 7.74^{a3}	59.68 ± 2.24^{c3}	54.76 ± 3.45^{d1}	0.001
P	0.047	0.038	0.001	0.987	

Different letters in the same row and different numbers in the same column show statistically different groups ($P<0.05$). BG: Bioactive glass, $Ca(OH)_2$: Calcium hydroxide

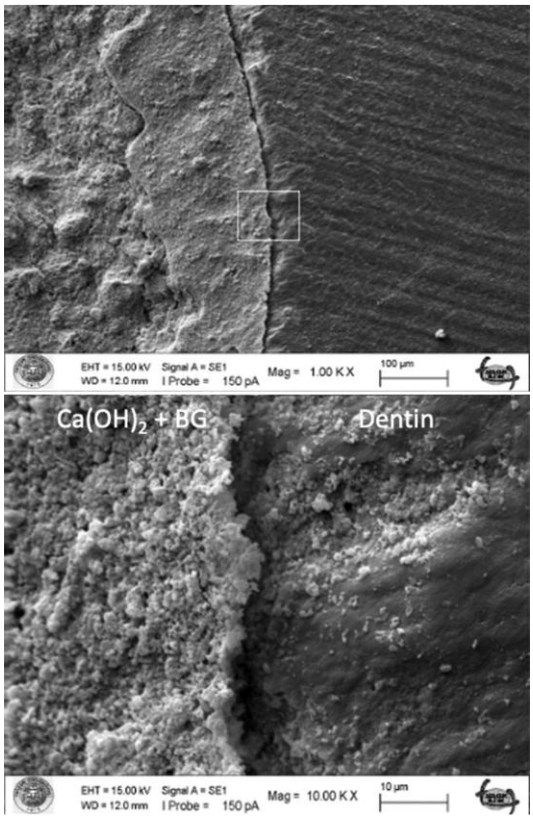
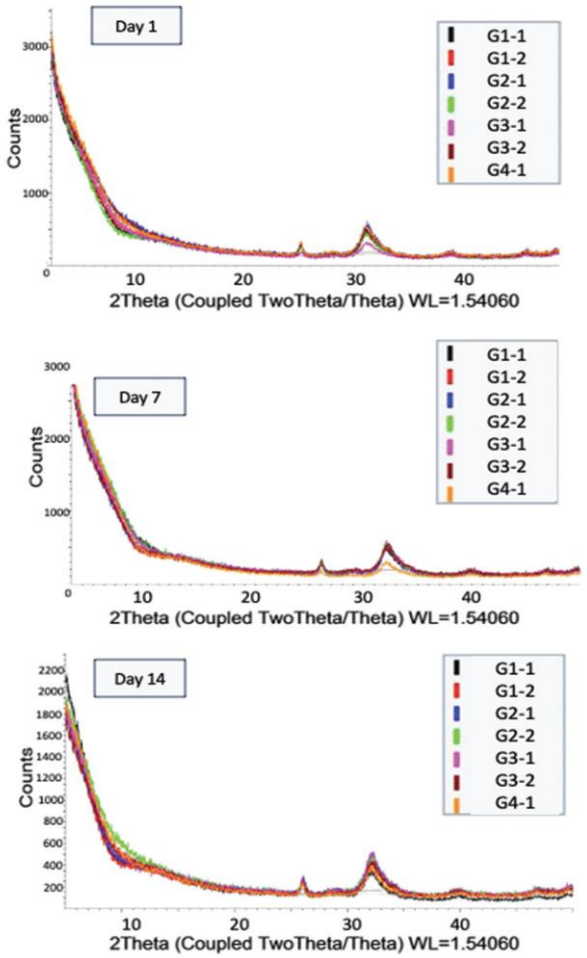


Figure 2: Scanning electron microscopy image of Ca(OH)2 + BG group at × 1000 and × 10,000 (The rectangular area at × 1000 image shows the dentin and medicament interface where the imaging was performed). SEM: Scanning electron microscopy, Ca(OH)2: Calcium hydroxide



XRD is a technique used to analyze crystalline materials, providing valuable information about their structure, composition, and physical properties.[22] The decrease in crystallinity observed in the $Ca(OH)_2$+ BG group may be due to the loss or substitution within the hydroxyapatite structure. The XRD results indicate that the BG group exhibits higher crystallinity in its hydroxyapatite lattice. In contrast, the samples treated with $Ca(OH)_2$+ BG for 14 days showed lower crystallinity and more substitutions in the hydroxyapatite lattice, likely due to the alkalinity of $Ca(OH)_2$. These results supported the hypotheses of Rosenberg *et al*. that $Ca(OH)_2$ may alter the mineral structure of root dentin, and that the strong alkalinity of $Ca(OH)_2$ may cause the collapse of the dentin structure – increased tooth fragility – by denaturing carboxylate and phosphate groups.[18] Although Kahler *et al*. found that $Ca(OH)_2$ is not associated with a reduction in the fracture strength of tested teeth even after 9 months,[23] a decrease in the mineral/matrix ratio and mineral crystallinity of radicular dentin was observed after exposure to $Ca(OH)_2$+BG in this study. The combination of these two medicaments may reduce the fracture strength of the dentin. In clinical practice, it is therefore important to control the duration of root canal medication with $Ca(OH)_2$+BG medicament. It is also necessary to perform a fracture strength study on the teeth related to $Ca(OH)_2$+BG medicament.

BGs have been suggested as potential intracanal drugs due to their ability to induce an alkaline pH[[24] similar to $Ca(OH)_2$-based drugs, stimulate bone tissue proliferation, and affect repair processes.[11] It has also been shown to have antimicrobial activity attributed to its pH.[24] *In vitro* studies have suggested that BG could be considered a potential substitute for $Ca(OH)_2$in intracanal therapy, owing to their similar spectrum of action and antimicrobial activity.[19,25] Carvalho *et al*. evaluated dentin pH by comparing the combinations of some different medicaments including $Ca(OH)_2$ and BG.[24] In the result of that study, $Ca(OH)_2$ showed significantly higher pH compared to the other groups at 30 days. BG presented an alkaline pH after 30 days.[24]The present study aimed to evaluate the pH of BG and $Ca(OH)_2$ when used separately or together at different time intervals, and their effect on the pH of dentin. Among the test groups, the $Ca(OH)_2$+BG combination had the highest pH values on the 1st, 7th, and 14th days ($P = 0.001$) [Table 1]. The group of samples that did not receive any medication had the lowest pH value. The highest pH result in the $Ca(OH)_2$+BG group after 14 days might be attributed to the alkaline-inducing properties of BG. As the antimicrobial activity of a material is also related to its pH,[24] the $Ca(OH)_2$+BG Combination may be recommended for use as a root canal medicament.

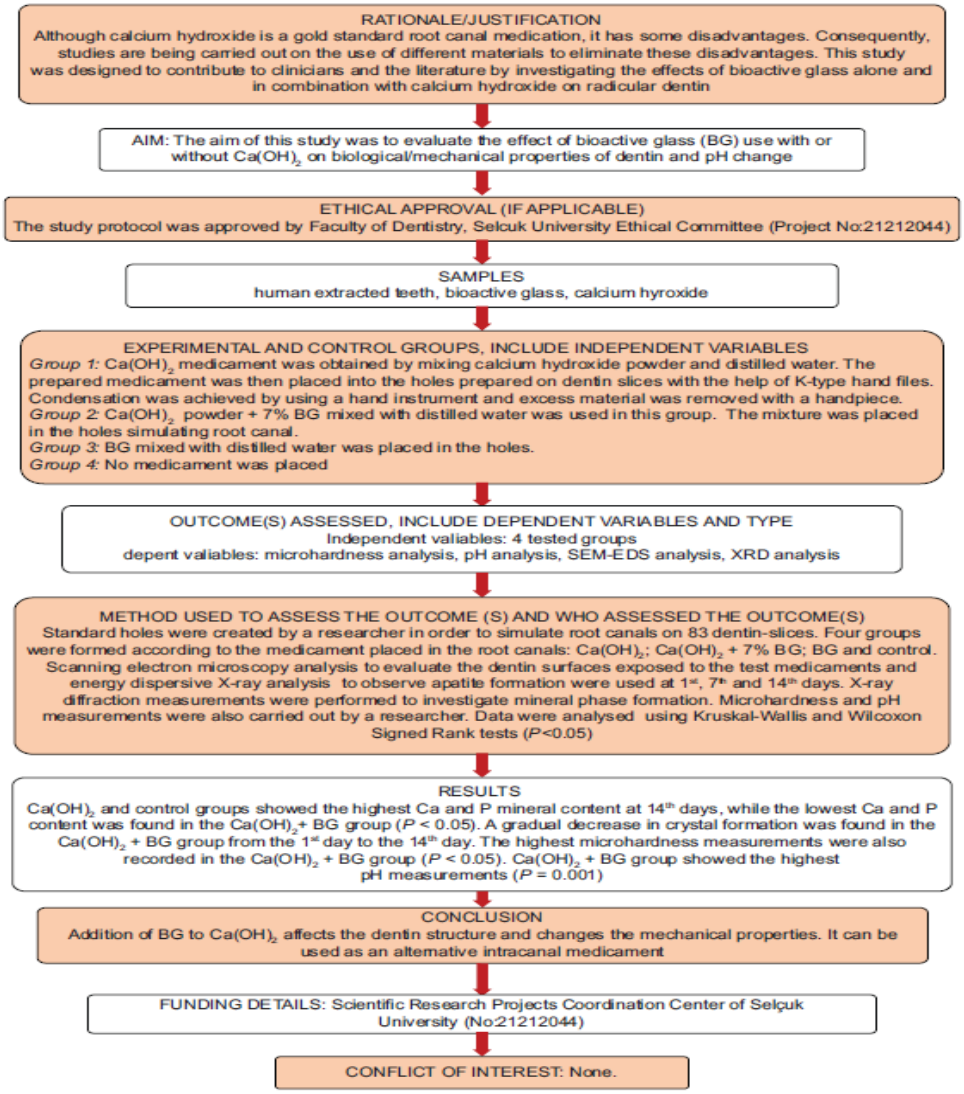


Figure 4: PRILE 2021 flowchart

Some intracanal medicaments can negatively impact the physical properties of root dentin. A decrease in dentin microhardness indicates the formation of softer dentin.[26] In this study, the highest microhardness values were observed in the $Ca(OH)_2$ group at the end of the 1st and 7th days, and in the $Ca(OH)_2$+BG group at the end of the 14th day [Table 2]. The lowest microhardness values were recorded in the control group across all experimental periods. Given the higher microhardness measurements, it can be concluded that the addition of BG to $Ca(OH)_2$ provides an advantage, positively affecting the dentin structure. In response to the research question of this study, it was demonstrated that both $Ca(OH)_2$ and BG can alter the mineral structure of dentin, even though the microhardness measurements were taken from surfaces that were not in direct contact with the medicaments.

This study has several limitations. The first is that nano-sized BG was not used, and the use of nano-BG might have resulted in different effects on the dentin structure. Additionally, various percentages of BG particles were not tested; only 7% of BG was used based on a previous study. Future research could explore the use of different BG concentrations and combinations to better understand their effects.

CONCLUSIONS

Within the limitations of this *in vitro* study, the combination of BG and $Ca(OH)_2$ affects the dentin structure and changes the mechanical properties. It can be used as an alternative intracanal medicament.

Conflicts of interest

There are no conflicts of interest

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