



Article

Effects of Adding Cinnamon, ZnO, and CuO Nanoparticles on the Antibacterial Properties of a Glass Ionomer Cement as the Luting Agent for Orthodontic Bands and Their Cytotoxicity

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Abstract: This study was conducted to evaluate the effects of adding cinnamon nanoparticles (NPs), Zinc oxide (ZnO) nanoparticles (NPs), and Copper oxide (CuO) NPs on the antibacterial property of a luting and lining glass ionomer cement (GIC) that was used for the cementation of orthodontic bands to the tooth. Cinnamon NPs, ZnO NPs, and CuO NPs were added into a luting and lining GIC in weight percentages of 1%, 2%, and 4%, respectively while a non-modified GIC was considered as the control group. Agar disc diffusion test was applied to assess the antimicrobial property of samples against *Streptococcus mutans* (*S. mutans*). The cytotoxicity of the nanoparticles was examined through the MTT assay for gingival fibroblasts. Data showed that GIC containing cinnamon and ZnO NPs displayed a larger inhibition zone diameter and greater antibacterial activity against *S. mutans* than CuO NPs. Meanwhile, there were no significant differences in the inhibition zone diameter of cinnamon NPs and ZnO NPs. The cytotoxicity assessment revealed the lower cytotoxicity of cinnamon NPs and the higher cytotoxicity of CuO NPs while the cytotoxicity of ZnO NPs was observed to be higher than cinnamon NPs and lower than CuO NPs. GIC containing cinnamon NPs exhibited noticeable antibacterial activity against *S. mutans* and cinnamon NPs revealed less cytotoxicity and it can be labeled as a favorable option for further assessment to be applied in fixed orthodontic treatments for the cementation of bands to teeth.

Keywords: cinnamon; ZnO; CuO; *Streptococcus mutans*; glass ionomer; cytotoxicity; nanoparticles

1. Introduction

The orthodontic band has been one of the most important components of fixed orthodontic treatments since the beginning of this specialty in the late 19th century [1]. One of the disadvantages of the orthodontic bands is that their application may lead to higher enamel demineralization, even more than bonded brackets. Its more posterior positioning in the mouth causes difficulties in the process of tooth brushing and eventually results in plaque accumulation [2,3]. According to relevant studies, the occurrence of white spot lesions (WSL) in the course of fixed orthodontic treatment is observed with an incidence and prevalence rate of 45.8% and 68.4%, respectively [4,5]. Glass ionomer cements (GICs) are one of the best choices for the cementation of orthodontic bands due to its biocompatibility, chemical adhesion to teeth, and fluoride ion release and uptake by enamel and dentin. Since the introduction of GICs to the markets, glass-ionomer cements (GICs) have shown the most potent anticaries tendency among the dental materials owing to its fluoride-releasing ability and remineralization potential [6]. However, this cement

can display a very low rate of activity against microorganisms upon the appearance of local biofilm accumulation, especially in fixed orthodontic conditions [7–9]. One of the solutions to this disadvantage is the addition of antibacterial nanoparticles into the glass ionomer to increase the antimicrobial activity of the cement. Various studies have focused on improving the antibacterial, remineralizing, and mechanical properties of GIC by adding CPP-ACP, bioactive ceramic particles, glass powders, and other chemical materials into the GIC powder [10–14].

The difference between nano materials and their counterpart bulk materials is their uniquely large surface-to-volume ratio that is provided by their small sizes and which can facilitate intimate interactions with microbial membranes and a considerably larger surface area for antimicrobial activity, offering a substantial antimicrobial property over other particles with the same composition [15,16]. One of the challenges of using nanoparticles is the maintenance of human health, since many researchers believe that their enhanced properties and nano-size may change the biological metabolic pathways of human cells and ultimately lead to the incidence of cancers and other diseases.

Nowadays, researchers are focused on the application of different plants, traditional substances, and natural products such as spices and herbal extracts against cariogenic bacteria such as *Streptococcus mutans* (*S. mutans*) [17]. As an anti-cariogenic material, there are several advantages to the usage of cinnamon due to its anti-inflammatory, cardioprotective, antioxidative, and antimicrobial properties. It can function as a promising antimicrobial agent in dentistry and be exerted in the production of mouth rinses, toothpastes, or root canal irrigants [18]. The results of previous studies indicated a noticeable antibacterial activity of cinnamon nanoparticles (NPs) against *S. mutans* [17].

Many studies have confirmed the potent antimicrobial feature of zinc oxide NPs, which also proved to be effective against a wide range of Gram-negative and Gram-positive bacteria and capable of inhibiting the formation of biofilm through antifungal activities [19]. The literature data indicate that the addition of ZnO NPs to dental materials can improve their antibacterial activity against cariogenic bacteria [20,21]. The nano-sized structure of these nano particles can diffuse into the microbial cell membranes and facilitate change their metabolic pathways, which would consequently annihilate or cause a decrease in microbial populations [22]. Next to the exhibition of antimicrobial activity by Copper oxide (CuO) against a wide range of pathogenic bacteria, there are reports on the noticeable antimicrobial effects of CuO NPs on the colonization and plaque formation of oral pathogens [23,24]. Recently, the attention of many researchers has been towards the incorporation of ZnO NPs, CuO NPs, and cinnamon NPs into dental materials. On the other hand, some herbal compounds, such as cinnamon NPs, have proven to be economically beneficial in addition to providing high antibacterial properties. Therefore, this study attempted to synthesize and add cinnamon NPs into a luting and lining GIC (which was used for the cementation of orthodontic bands). We also performed a comparison between cinnamon NPs-containing GIC antibacterial property and ZnO NPs-containing GIC and CuO NPs-containing GIC to investigate their effects in the course of reducing dental caries in orthodontic banding. To the best of our knowledge, there have been no assessments on the impacts of the addition of these nanoparticles on the antimicrobial properties of conventional luting and lining GICs up to this date. As the last step, we investigated the cytotoxicity of each of the three nanoparticles and presented the gathered outcomes.

2. Materials and Methods

To begin the process, cinnamon NPs were produced through a hydrothermal synthesis method. For this purpose, 0.3 g of ground cinnamon powder was weighed to be mixed with 100 cc of distilled water. Then, the resulting mixture was thoroughly dispersed to be transferred into an autoclave and placed in the oven at 180 °C for 4 h. Once the resultant product was cooled down, it was centrifuged and freeze-dried to produce the powder of cinnamon nanoparticles. The obtained cinnamon NPs were examined by the application of a Transmission Electron Microscope (TEM) (Leo 912 AB, Carl Zeiss, Oberkochen, Germany).

Different weight percentages of 1%, 2%, and 4% of cinnamon NPs, ZnO NPs (US Research Nanomaterials, Houston, TX, USA), and CuO NPs (US Research Nanomaterials, Houston, TX, USA) were added into a luting and lining GIC (Gold label, GC Corporation, Tokyo, Japan), while a non-modified GIC was considered as the control group. Three disk-shaped samples were prepared for each group by the usage of a mold with a height of 3 mm and a diameter of 6 mm. Agar Disc diffusion test was exerted to assess the antimicrobial property of samples. The disks were placed on the Mueller Hinton Agar (MHA) that was inoculated with 0.5 McFarland of *S. mutans* (Persian Type Culture Collection, Tehran, Iran). Subsequent to being incubated at the temperature of 37 °C for 24 h, the antimicrobial activity of the specimens was evaluated by determining the diameter of clear inhibition zone that surrounded the specimen discs.

In this study, we evaluated the effects of different concentrations of each nanoparticle on human gingival fibroblasts. Initially, gingival fibroblasts were cultured in culture media with high glucose (GIBCO, Invitrogen), 1:100 streptomycin/penicillin, and 10% HI-FBS. In brief, the cells were seeded into a 96-well plate at 5000 cells/well to be cultured overnight, while containing 100 µL aliquots of growth medium, which were then incubated for 48 h with different concentrations of nano particles in triplicate. Subsequently, 5 mg/mL of MTT solution was added to every plate to be incubated at 37 °C for 4 h as the last step. The addition of 100 µL of Dimethyl Sulfoxide solution was considered to dissolve the Formosan crystals. Then, a microplate reader (Anthos, Australia) was used to measure the absorbance at 570 and 630 nm. Once the middle-maximal inhibitory concentration (IC₅₀) of every group was calculated, the concentration of NPs that reached 50% of cell annihilation with each compound was also determined and, thus, the IC₅₀ was calculated from the cell mortality curves.

3. Results

In conformity to the exhibited TEM image and size distribution of cinnamon nanoparticles in Figure 1, the average size of these particles was observed to be 153.5 nm.

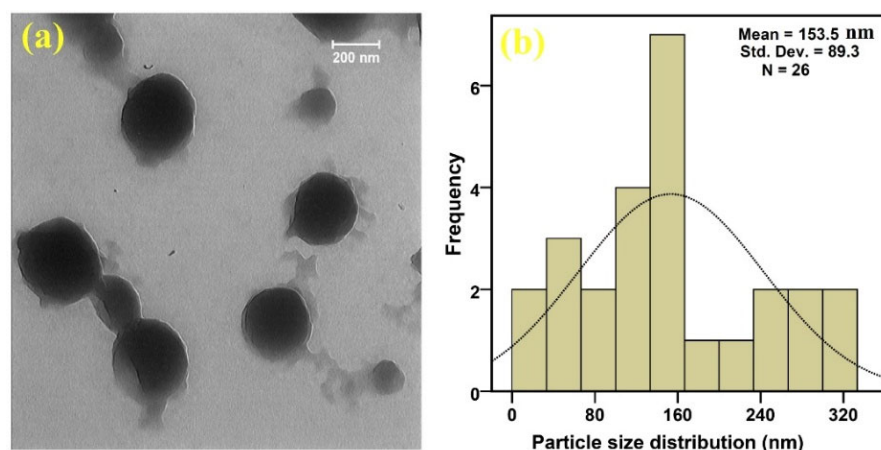


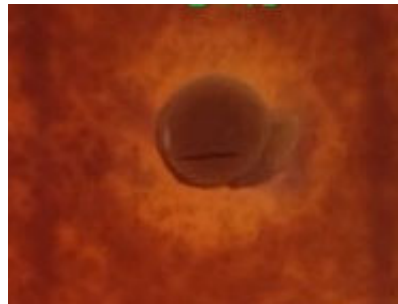
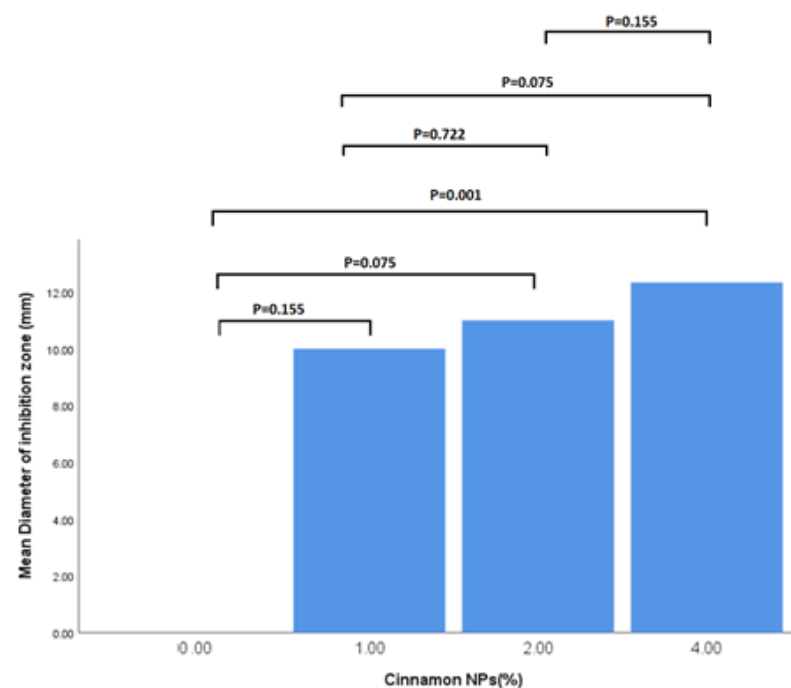
Figure 1. (a) TEM image, (b) size distribution of cinnamon NPs.

The results of the addition of cinnamon NPs and their effects on the antibacterial properties of glass ionomer cement are displayed in Table 1, which also presents the diameter of inhibition zone of *S. mutans* bacteria (Figure 2).

The outcomes of the Kruskal–Wallis test indicated the existence of a significant difference between the groups ($P < 0.05$). Figure 3 provides the results of the performed Mann–Whitney pairwise comparison test on the groups. As can be observed, the addition of 4% cinnamon NPs caused a significant increase and achieved the highest inhibition zone diameter and also empowered the antibacterial property against *S. mutans* bacteria.

Table 1. Diameter of inhibition zone of *S. mutans* bacteria for GIC in different weight percentages of cinnamon NPs.

Groups	Mean (mm)	Standard Deviation	Standard Error
GIC	0	0	0
GIC + 1% Cinnamon NPs	10	1.73	1
GIC + 2% Cinnamon NPs	11	0	0
GIC + 4% Cinnamon NPs	12.33	0.57	0.33

**Figure 2.** Inhibition zone of *S. mutans* bacteria for GIC containing cinnamon NPs.**Figure 3.** The result of the pairwise comparison test on the groups for cinnamon NPs addition into the GIC.

The results of measuring the diameter of inhibition zone (Figure 4) for the CuO NPs-containing GIC are demonstrated in Table 2. The Kruskal–Wallis test showed that there was a significant difference between the groups ($P < 0.05$).

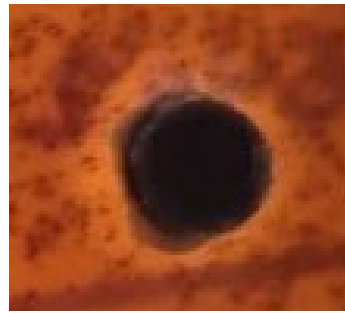


Figure 4. Inhibition zone of *S. mutans* bacteria for GIC contained CuO NPs.

Table 2. Diameter of inhibition zone of *S. mutans* bacteria for GIC in in different weight percentages of CuO NPs.

Groups	Mean (mm)	Standard Deviation	Standard Error
GIC	0	0	0
GIC + 1% CuO NPs	10	0	0
GIC + 2% CuO NPs	10.33	1.55	0.66
GIC + 4% CuO NPs	11	0	0

The outcomes of the Mann–Whitney pairwise comparison test are shown in Figure 5. The GIC that contained 4% of CuO NPs displayed the largest diameter of the inhibition zone and antimicrobial properties against *S. mutans* bacteria.

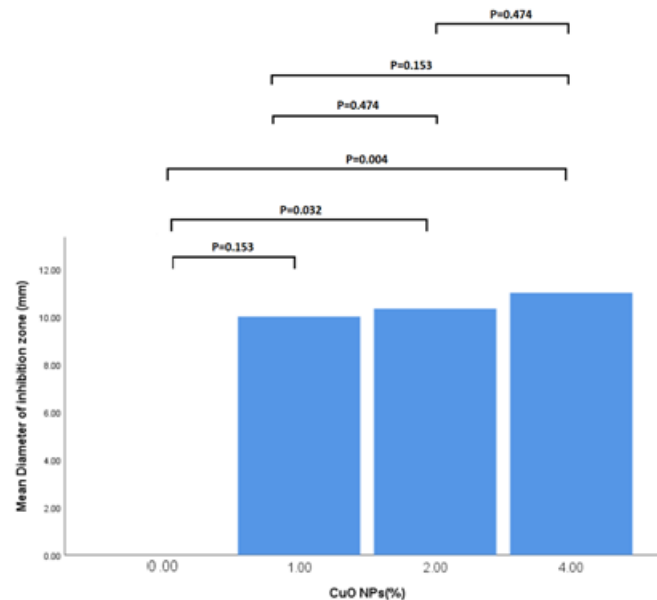
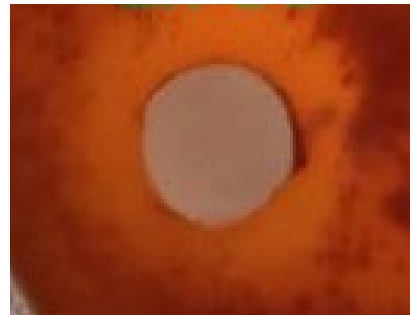


Figure 5. The results of the pairwise comparison test on the groups for addition of CuO NPs into the GIC.

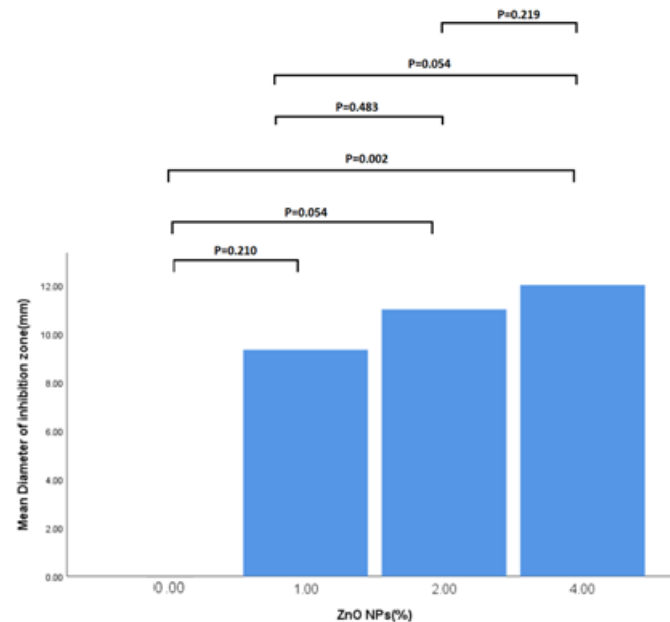
Table 3 shows the effects of incorporating ZnO NPs on the diameter of the inhibition zone (Figure 6). The outcomes of the Kruskal–Wallis test were indicative of a significant difference between the groups ($P < 0.05$).

Table 3. Diameter of the inhibition zone of *S. mutans* bacteria for GIC in different weight percentages of ZnO NPs.

Groups	Mean (mm)	Standard Deviation	Standard Error
GIC	0	0	0
GIC + 1%ZnO NPs	9.33	1.53	0.88
GIC + 2% ZnO NPs	11	0	0
GIC + 4% ZnO NPs	12	0	0

**Figure 6.** Inhibition zone of *S. mutans* bacteria for GIC containing ZnO NPs.

The results of the Mann–Whitney pairwise comparison test are presented in Figure 7, which displays the inducement of a significant increase in the diameter of inhibition zone against *S. mutans* bacteria upon the addition of ZnO NPs up to percentage of 4%.

**Figure 7.** The results of the pairwise comparison test on the groups for the addition of ZnO NPs into the GIC.

The Kruskal–Wallis test was indicative of a significant difference in the inhibition zone diameter of glass ionomers that contained cinnamon, CuO, and ZnO NPs at the percentage of 4%, which resulted in achieving the largest diameter of inhibition zone ($P < 0.05$). Moreover, the Mann–Whitney pairwise comparison (Table 4) of GIC containing cinnamon and ZnO NPs displayed a larger inhibition zone diameter and greater antibacterial activity against *S. mutans* than CuO NPs. Meanwhile, there was a lack of any significant differences in the inhibition zone diameter of cinnamon NPs and ZnO NPs ($P = 0.617$).

Table 4. The results of the pairwise comparison test on the groups for the addition of 4% cinnamon, 4% CnO, and 4% ZnO NPs into the GIC.

(I) Group	(J) Group	P-Value
GIC + 4% cinnamon NPs	GIC + 4% CnO NPs	0.012
	GIC + 4% ZnO NPs	0.617
GIC + 4% CuO NPs	GIC + 4% ZnO NPs	0.046

The results of cytotoxicity assessment of the three nanoparticles obtained by the means of MTT assay for gingival fibroblasts are presented in Figures 8–10.

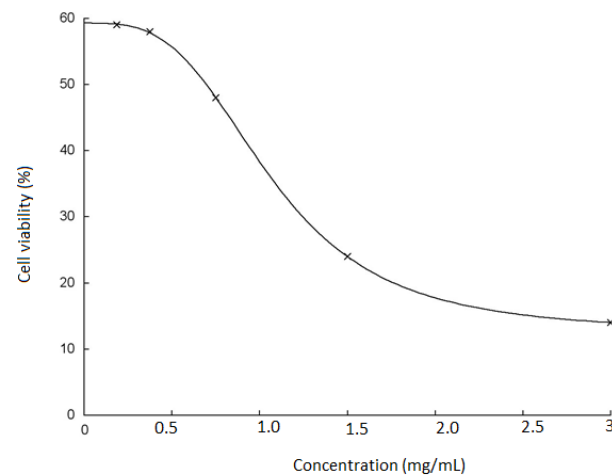


Figure 8. Graph of the percentage of cell viability of gingival fibroblasts in different concentrations of cinnamon NPs.

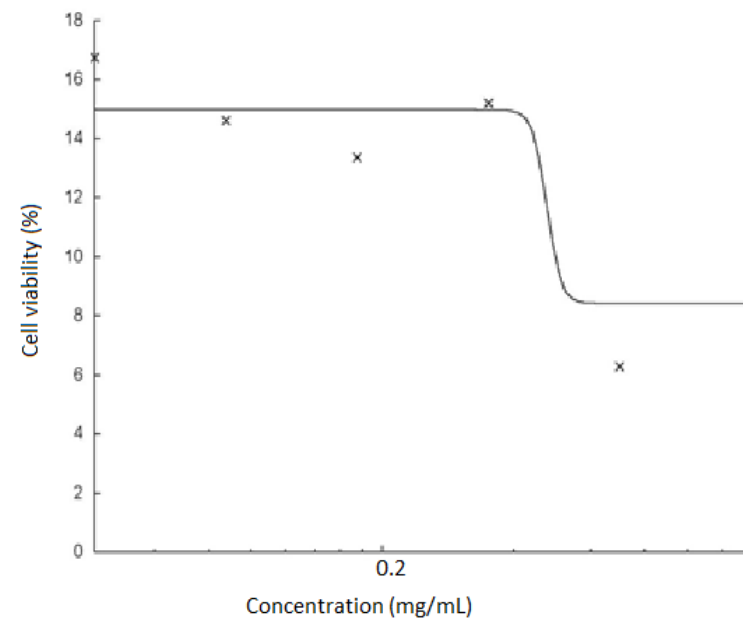


Figure 9. Graph of the percentage of cell viability of gingival fibroblasts in different concentrations of CuO NPs.

According to figures, the IC₅₀ value of cinnamon NPs, ZnO NPs, and CuO NPs were 1060, 460, and 380 µg/mL, respectively. In other words, cinnamon NPs induced a lowest cytotoxicity and CuO NPs displayed a highest cytotoxicity, while the cytotoxicity of ZnO NPs was higher than cinnamon NPs and lower than CuO NPs.

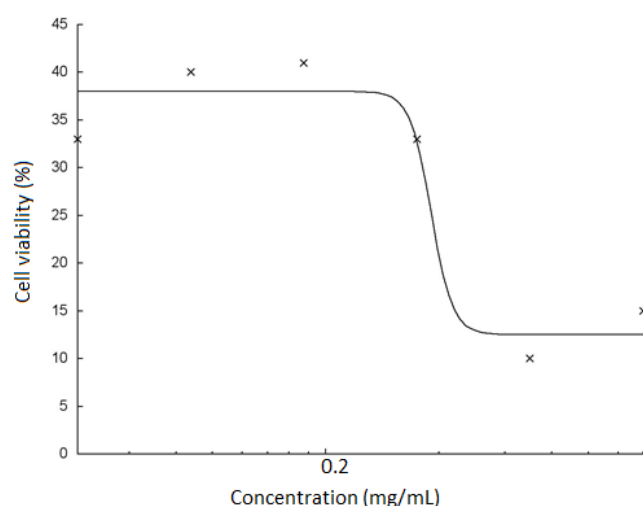


Figure 10. Graph of the percentage of cell viability of gingival fibroblasts in different concentrations of ZnO NPs.

4. Discussions

For the very first time, this study attempted the addition of cinnamon NPs to the GIC that was used for the cementation of orthodontic bands to the teeth. Although the results indicated the effectiveness of each GIC that contained cinnamon, ZnO, and CuO NPs against *S. mutans*, the samples of cinnamon and ZnO NPs displayed a larger inhibition zone diameter and consequently more antibacterial activity than CuO NPs.

According to the outcomes, the addition of 4% cinnamon NPs induced a significant increase and achieved the highest inhibition zone diameter and also exhibited the strongest antibacterial property against *S. mutans* bacteria. Generally, the antimicrobial property of cinnamon NPs is caused by the presence of Cinnamaldehyde, Eugenol, benzoic acid, and cinnamic acid in this material [25–27]. The in vitro study of Yaseen et al. [17] investigated the effect of adding 1% and 3% cinnamon nanoparticles to the orthodontic composite that was used for the bonding of brackets to teeth. As a result, they observed the inducement of a significant antimicrobial effect by the composite that contained 3% of cinnamon NPs against *S. mutans* bacteria. Their experimental cinnamon NPs were produced through the mechanical attrition method; however, this study exerted a hydrothermal method for the production of cinnamon NPs. In conformity to the report of Zainal-Abidin et al. [28], the minimal inhibitory concentration of cinnamon oil against cariogenic bacteria was in the range of 0.21 to 0.63 mg/mL. Additionally, Bersy et al. [29] investigated the antibacterial effect of a mouthwash that contained cinnamon in children with an age range of 6 to 12 years, which resulted in reducing the *S. mutans* population in saliva by 96.99% after 30 min.

The outcomes of present study confirmed the ability of GIC containing 4% of ZnO NPs in achieving the largest inhibition zone diameter and displaying the strongest antimicrobial properties against *S. mutans* bacteria in different percentages. ZnO NPs can destroy the cell wall and produce reactive oxygen species (ROS) such as hydrogen peroxide to prevent the growth of bacteria and result in their death. According to the report of Malekhoseini et al. [20], the addition of ZnO NPs to the resin modified glass ionomer Fuji II LC resin (GC Corporation, Tokyo, Japan) caused a significant increase in its antibacterial property against *S. mutans*. Moreover, the study of Vanajassu et al. [30] indicated that the addition of 3% ZnO NPs to Fuji IX GIC (GC, Tokyo Japan) led to the exhibition of a significant antibacterial property.

Considering the different percentages of CuO NPs, the results of our study determined that the glass ionomer cement with 4% of CuO NPs displayed the largest diameter of inhibition zone and the strongest antimicrobial property against *S. mutans* bacteria.

Regarding the antibacterial mechanism of CuO NPs, they were mentioned to release Cu^{2+} that can trigger the production of reactive oxygen species (ROS) within the bacteria

and result in cell injury and apoptosis [31]. The report of Toodehzaeim et al. [32] indicated that adding up to 1% of CuO NPs to Transbond XT orthodontic composite can cause significant antibacterial properties against *S. mutans* [33]. Additionally, the observations of Argueta et al. [34] suggested the antibacterial features of the orthodontic adhesive that contained CuO nanofillers against *S. mutans*, *S. sanguineus*, and *S. aureus* bacteria.

According to the comparison of our results of the three nanoparticles, cinnamon NPs displayed the least cytotoxicity and CuO nanoparticles induced the highest, while the cytotoxicity of ZnO NPs was less than CuO NPs. Cinnamaldehyde is known as one of the main causes of cinnamon cytotoxicity [35]. The discoveries of LeBel et al. indicated that the usage of effective concentrations against *S. moorei* induced a negligible loss of viability in gingival keratinocytes after 1 h of exposure, while Marcoux et al. [36] reported the observance of some cytotoxicity from cinnamon oil.

The outcomes of our study indicated that CuO NPs displayed a higher rate of cytotoxicity than the other two nanoparticles. A possible reason for the cytotoxicity of metal-based nanoparticles is their production of reactive oxygen species (ROS) that lead to oxidative stress [37]. In comparison to chlorhexidine (CHX), Eslami et al. [38] evaluated the biocompatibility of colloidal solutions that contained ZnO, CuO, titanium dioxide (TiO₂), and silver (Ag) NPs in a culture of human gingival fibroblasts. Their results showed that CHX and CuO-containing solution displayed the highest rate of apoptosis among the groups. Apparently, the cytotoxicity of CuO NPs, which is mainly caused by oxidative stress [33,39,40], occurs in human cell lines such as human lung epithelial A549, human cardiac microvascular endothelial, kidney, and neuronal cells.

Our data indicated that the cytotoxicity of ZnO NPs is higher than cinnamon NPs and lower than CuO NPs. Chen et al. [41] investigated the effect of ZnO NPs on the proliferation and toxicity of human gingival cells and observed the inducement of toxicity by the high concentrations of ZnO NPs in human gingival fibroblast cells. Furthermore, the discoveries of Wang et al. reported the lack of any damaging occurrence by ZnO NPs in human normal keratinocytes (HaCaT cells) and gingival fibroblasts (HGF-1 cells). In another study, Kim et al. [42] investigated and compared the toxicity of four different oxide nanoparticles (Al₂O₃, CeO₂, TiO₂, and ZnO NPs) on two cell lines and reported that ZnO NPs displayed the highest cytotoxicity in terms of cell proliferation, cell viability, membrane integrity, and colony formation in both cell lines. Ng et al. [43] investigated the in vitro and in vivo cytotoxicity and genetic toxicity of ZnO NPs in regards to human lung fibroblasts and fruit fly, which resulted in observing the annihilation of human lung fibroblasts by ZnO NPs. It was also noteworthy that oxidative stress damaged the DNA of these cells.

The limitations of this research are that the study was designed as an in vitro evaluation, which does not fully represent oral conditions. For more meaningful results, future in vivo studies could be performed. For example, a double-blind randomized clinical trial study could be carried out in patients who were candidates for fixed orthodontic treatment and the effects of the addition of these nanoparticles could be evaluated by the measurement of the *S. mutans* counts around the orthodontic bands through the usage of real-time PCR.

5. Conclusions

The noticeable antibacterial activity of the GIC that contained cinnamon NPs against *S. mutans* was confirmed to be much more superior to the exhibited properties by ZnO and CuO NPs-containing GICs. In addition, since cinnamon NPs revealed a considerably lower value of cytotoxicity than ZnO and CuO NPs, they constitute a favorable option for further research in regards to the potential application in fixed orthodontic treatments for the cementation of bands to teeth.

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writing—review and editing, H.K., A.R., H.S., M.D. and H.R.; supervision, A.R. and H.S.; project administration, H.S.; funding acquisition, H.S. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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