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Evaluating the Citotoxicity and Antibacterial Activity of Nano Zinc-Glyde Mixture for Intracanal Irrigation

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Abstract

Objective: In this research, we evaluated the antibacterial efficiency and cytotoxicity of Glyde including its Zinc NPs preparations in three different ratios comparing them with the conventional Glyde. **Material and Methods**: Antibacterial efficiency was evaluated using inhibition zone method against three common gram-positive pathogens *Enterococcus faecali, Lactobacillus and Streptococcus.* Three different irrigant preparations were evaluated, GI: Glyde gel alone as a control group, GII: experimental gel (3% Zinc NPs-Glyde), GIII: (4% Zinc NPCs-Glyde gel), GIV: (5% Zinc NPCs-Glyde gel). We evaluated the cytotoxicity by the MTT test. assessing the cells' viability as soon as possible after 24h, 48h, and 72h. After a color change assessment, spectrophotometric analysis with wavelength ranged from 190-780 nm was performed. The spectrum analysis was performed for diluted mixtures in solvent. Data were plotted and recorded for each wavelength. Kruskal–Wallis ($p < 0.05$) and post-hoc Bonferroni pairwise ($p < 0.05$) were used for statistical analysis. **Results**: All the groups a reduction of the three types of bacteria ($p > 0.05$) was seen, with inhibition zone increasing with increasing addition of zinc NPs up to 5%. For cytotoxicity, it seems that cell bioavailability remained for 24h, 48h, and declined at 72h. Data were not correlated with addition of zinc nano particles, especially within the visible light range. **Conclusion**: The addition of zinc nanoparticles has acceptable antibacterial properties and cytotoxic features, and Glyde gel may be used for root canal disinfection without remarkable color change.

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Introduction

In most cases of irreversible pulpitis and pulp necrosis, pathogens get into the pulp through mainly caries, periodontal disease or trauma [1,2]. Dahlén et al. [3] suggested that this kind of inflammation is often caused by specific bacterial strains such as *lactobacilli, streptococci*, and gram-negative obligate anaerobes. The root canal system is shaped and cleaned chemically and mechanically for the removal of inflamed and/or necrotic pulp tissue, and thus, of established bacteria. Chemical cleaning has been recommended for the root canal system [4]. Sodium hypochlorite works well at amounts between 0.5 and 5.25% [5-7].

Zinc oxide nanoparticle (ZnO NP) is an odorless white powder found in bone, muscle, skin, and teeth. Its molecular weight is 81.38 g/mol and the U.S. Food and Drug Administration (FDA) recognized it as a safe substance (GRAS). The use of zinc oxide nanoparticles (ZnO NPs) in biomedicine is safe, show no systemic toxicity, and are cost-effective based on their increased specific surface area and enhanced particle surface activity [7, 8].

Jowkar et al. found that adding ZnO to an EDTA solution for irrigation enhanced the fracture resistance of the roots [9,10]. A study by Aguiar et al. discovered that these NPs helped with alkalization and acted against *E. faecalis* when mixed with calcium hydroxide NPs and chlorhexidine [11].

Material and Methods

Chemicals and reagents

Glyde Gel (Dentsply-Sirona, Switzerland) was used as a base material in this experiment. The nano zinc with high purity of 99.9 %, 35-45 nm, metal base applied in this work was supplied from US Research Nanomaterial's, Inc. 3302 Twig Leaf Lane, Houston, TX77084, USA.

Glyde-nano zinc mixture was prepared in three different loading percentages 3%, 4 % and 5 %. All preparations were performed under sterile conditions to avoid any samples contaminations. The three experimental mixtures (group II: III, and group IV) were prepared by weighting zinc nanoparticles following by vigorous mixing with glyde to prepare exactly the required percentage of

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mixture of Zn nano-Glyde. Group I represent the glyde material without any additive nano particles to serve as comparison.

Antibacterial activity test

Three different human pathogenic bacteria, *Enterococcus faecali*, (a gram-positive, facultative anaerobic coccus), *Lactobacillus* (Gram-positive, Obligate anaerobes rods) and *Streptococcus (*Gram-positive Facultative anaerobes cocci) were investigated as tested organisms. A clean cotton swab was used to slide some of the bacterial solution around on the Muller-Hinton agar medium and make sure it was spread out evenly. After that, the mix was left for 10 minutes. In the layer of agar that came before, 3 wells with a thickness of 5 mm were made. Each well got 50 microliters of purified and crude EPS, and D.W. was put in the middle well as a control. After that, the agar plates were taken out. For 18 hours, the plates were kept at 37 °C. Then, the inhibition zones' widths were recorded. The well diffusion experiment was used to see if glyde-zinc nanoparticles could kill pathogens. In each well of the Petri plates, three different amounts of Nano zinc mix were added: 3%, 4%, and 5%.

MTT assay

The neutral red (NR) and tetrazolium MTT in vitro cytotoxicity assays were compared for prepared mixtures of glyde – nano zinc mixtures at maximum loading (5%) and for the glyde only on fibroblast cell line as the bio indicator, at three-time intervals (24hr, 48h, and 72h). There was good agreement (r = 0.9052) in how the test agents were ranked based on their median cytotoxicity values (NR50 and MTT50), even though the tests were based on different physiological endpoints.

Spectroscopic analysis

Glyde-Nano zinc mixtures were scanned using spectrophotometer. The wavelength ranged from 190-780 nm. The expert data were plotted and record in each wavelength.

Statistical evaluation

The three groups were compared using the Kruskal–Wallis test ($p < 0.05$): GI (glyde alone), GII (glyde plus 3% zinc), GIII (glyde plus 4% zinc), and GIV (glyde plus 5% zinc). We used Post-hoc Bonferroni pairs ($p < 0.05$) to find the means that are very different from each other.

Results

Table 1 shows the inhibition zones as antibacterial test against *Lactobacillus* (Gram-positive, obligate anaerobes rods). There was a significant difference depending on the added zinc nanoparticles with different ratios of glyde as intracanal material ($p > 0.05$). When using post hoc Bonferroni, we found a significant difference between GII and GIV.

In Table 2, inhibition zone diameters (mm) of *Enterococcus faecali*, (a gram-positive, facultative anaerobic coccus) by antibacterial action of Glyde groups showed a significant difference among tests groups by adding zinc nano particles ($p>0.05$). Post-hoc Bonferroni pairwise comparisons showed a significant difference between Glyde group alone and group IV (5% zinc).

In Table 3, there was a significant difference among tests groups ($p>0.05$) according to the inhibition zones diameters (mm) of Streptococcus (Gram-positive Facultative anaerobes cocci) by the effect of Glyde alone and Glyde mixed zinc nano particles. Using Post-hoc Bonferroni pairwise comparisons showed a significant difference between Glyde group alone and group IV (5% zinc).

To evaluate cytotoxic, a human normal fibroblast cell line (BJI) was used. The MTT essay was performed at 24h, 48h and 72h. At 24h, the result revealed that Glyde alone IC50 was at a dose of 85.6mg/ml. After adding zinc nanoparticles within the same interval, the IC50 was 83.12mg/ml. Cell viability was 92.66% at a dose of 50mg/ml, while at the same dose cell viability was 77.16% with Glyde. Increasing the dose of MTT up to 1000mg/ml decreased cell viability for both Glyde and Glyde zinc mix (10.94% and 11.15%, respectively) (Figures 1 and 2).

At 48h the result revealed that Glyde alone IC50 was at a dose of 85.6mg/ml. After adding zinc nanoparticles within the same time, the IC50 was 83.12mg/ml. Cell viability was 92.66% at a dose of 50mg/ml for Glyde only, while at the same dose, cell viability was 77.16% for Glyde zinc mix. Increasing the dose of MTT up to 1000mg/ml decreased cell viability for both Glyde and Glyde zinc mix (10.94% and 11.15%, respectively) (Figures 3 and 4).

At 72h, the result revealed that IC50 was 44.71 mg/ml for Glyde alone and 7.71mg/ml for Glyde zinc mix. On the other hand, cell viability at a dose of 50mg/ml was 40.12% for Glyde alone and 27.17% for Glyde zinc mix. When the dose was increased up to 500mg/ml, only 7.25% viability was seen for Glyde only and 5.85% viability was seen at a dose of 1000mg/ml (Figures 5 and 6).

Spectroscopic analysis

The UV-VIS scanning recorded very similar values, without any intense abnormal peaks, especially in visible range.

Discussion

Nanoparticles are often used to combat microbial infections because they are less cytotoxic [12,13]. To test the antibiotic effects of added zinc nanoparticles to Glyde, Enterococcus *faecali* was picked because it is one of the types most often found in individuals who have had persistent root canal infections. Another reason, *E. faecalis* can live in harsh conditions with few nutrients and for a long time inside canals, even after treatment [14,15]. It can also survive at very hot temperatures or acidic conditions [16].

In our study, selection of *Streptococcus* and *lactobacillus* species was due to their virulence and involvement in rapid progression of periodontal diseases, endoperio lesions, both acidogenic and aciduric environments, enamel demineralization leading to dental caries, and initiation of biofilm containing *Streptococcus* [17-19].

The antibacterial effect of Glyde gel depends on the oxygen bubbling which are liberated from carbamide peroxide through the removing of pulp tissue [20]. As shown in Tables 1, 2, and 3 there was a significant difference among tests groups ($p>0.05$) according to the inhibition zones diameters (mm) for the three bacterial types. Results indicated that as we increase the percentage of zinc nanoparticles in the mixtures, the more inhibition zones size appeared in diameter for the three types of bacteria in comparison with the Glyde alone or even low zinc percent. These results may relate to electrostatic forces between the positive bacterial cell wall and the positive NPs that enhance the antibacterial activity [21]. Positively charged nano particles showed higher antibacterial activity against both *S. mutans and E. faecalis*. While for the first time zinc nano mixtures was investigated for its antibacterial efficiency, many other metal nano particles revealed same higher activity against different types of bacteria when added to Glyde [22]. Or they filled the canals with nanoparticles as a kind of irrigant [16]. Our study looked at the possibility of harmful cytotoxicity of a Glyde material (after 24 h, 48 h, and 72 h) with and without 5% zinc nanoparticles as the highest loading percent. To get to a level that is useful in medicine, we used the MTT method to look for deadly activity in the human normal fibroblast cell line (BJI). Color was used in the MTT test to show how metabolically active cells are and how dangerous they are. This is by turning a yellow color called 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide that dissolves in water into purple formazan crystals that do not dissolve in water [23]. Material considers as cytotoxic if it kills more than half of the cell's lysis, which is a score of ≤ 21. As per ISO 10993-5, the test extract was harmful if less than 70% of the cultures survived compared to control cultures that were not handled with it [24,25].

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Immediately after 24h, the findings revealed that there was a remarkable difference for adding zinc Nano particles to Glyde material when IC50 diminished from 220.1 to 56.03 mg/ml but cell viability was still acceptable for both Glyde and Glyde zinc mix, even when decreased, mostly due to the immediate role of zinc particles. Up to 48 h, we saw that both Glyde and its zinc additive mixture reached close to IC50 results with cell viability at 50 mg/ml. MTT dose of 92.66473274%, 77.16150079%, which is suitable biocompatibility. While increasing time up to 72h, we noticed cell viability increasing by adding zinc nano particles compared with Glyde alone. This could be due to suppression of cytotoxicity by interaction between Glyde EDTA and zinc nano particles [26]. Considering the increasing demands for aesthetics, biomaterials should be chromatically stable, present optical properties like dental structures and not exert staining effects to hard dental tissues [27]. Biomaterials should be color stable, have visual features like tooth structures, and not damage hard mouth tissues. The zinc mixture used in this study could not change color and zinc, a small metal, seen to cause staining. Zinc may even reverse discoloration and greatly reduces the darkening of teeth caused by SDF [28].

Conclusions

The study results revealed that zinc nano particles, when added to Glyde, significantly increased the antibacterial activity against three types of human pathogens: *Enterococcus faecali, Lactobacillus* and *Streptococcus.* It also showed it is antibacterial efficiency is dose dependent. However, this offers a promising material to be used during rotary instrumentation as a lubricant and antibacterial material at the same time. These experimental mixtures revealed favorable cell viability results when tested by MTT assay and they did not induce remarkable color change.

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Kruskal-Wallis Test				Post-hoc Bonferroni pairwise comparisons			
Lacto + Glyde mixed groups							
Group	N	Median (Range)	P-value	Sample 1 + Sample 2	Test Statistics	Standard Error	P-value
Lacto+ GII	3	21 (1)	0.02	Lacto +G II Lacto +GI	- 0.3	2.9	1.0
Lacto+ GIII	3	24 (2)		Lacto+ GII Lacto +GIII	- 4.6	2.9	0.6
Lacto+ GIV	3	28 (1)		Lacto +GII Lacto +GIV	- 7.6	2.9	0.05
Lacto+GI	3	22 (2)		Lacto +GI Lacto +GIII	4.3	2.9	0.8
				Lacto +GI Lacto +GIV	7.3	2.9	0.07
				Lacto + GIII Lacto +GIV	- 3.0	2.9	1.0

Table 1. Inhibition zone diameters in mm of *Lactobacillus* bacteria by Glyde alone and mixed groups.

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Enterococcus faecali +Glyde mixed groups							
Group	N	Median (Range)	P-value	Sample 1 – Sample 2	Test Statistics	Standard Error	P-value
Entero+ GII	3	20 (2)	0.03	Entero + GI Entero + GII	2.1	2.8	1.0
Entero + GIII	3	22 (3)		Entero + GII Entero + GIII	- 1.6	2.9	1.0
Entero + GIV	3	25 (1)		Entero + GII Entero + GIV	- 5.8	2.9	0.2
Entero + GI	3	20 (1)		Entero + GI Entero + GIII	3.8	2.9	1.0
				Entero +GI Entero +GIV	8.0	2.9	0.02
				Entero +GIII Entero +GIV	- 4.1	2.9	0.8

Table 2. Inhibition zone diameters in mm of *Enterococcus faecali* bacteria by Glyde alone and mixed groups.

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Strepto+Glyde mixed groups							
Group	N	Median (Range)	P-value	Sample 1 – Sample 2	Test Statis-tics	Standard Error	P-value
Strepto+ GII	3	15 (1)	0.01	Entero +GI Entero +GII	2.1	2.8	1.0
Strepto + GIII	3	22 (1)		Entero +GII Entero+ GIII	- 1.6	2.9	1.0
Strepto + GIV	3	24 (1)		Entero +GII Entero + GIV	- 5.8	2.9	0.2
Strepto + GI	3	13 (1)		Entero + GI Entero + GIII	3.8	2.9	1.0
				Entero +GI Entero +GIV	8.0	2.9	0.02
				Entero +GIII Entero + GIV	- 4.1	2.9	0.8

Table 3. Inhibition zone diameters in mm of *Streptococcus* bacteria by Glyde alone and mixed groups.

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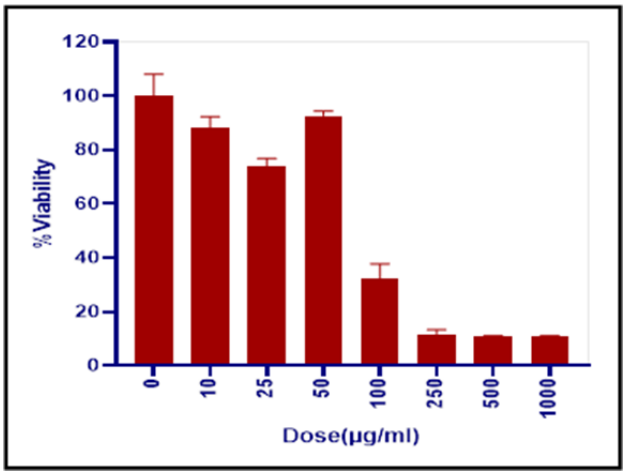

Figure 1. Cell availability of Glyde alone at 24h.

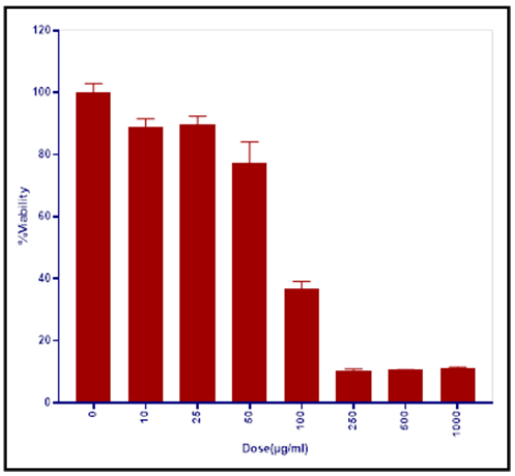

Figure 2. Cell availability of Glyde – zinc mix at 24h.

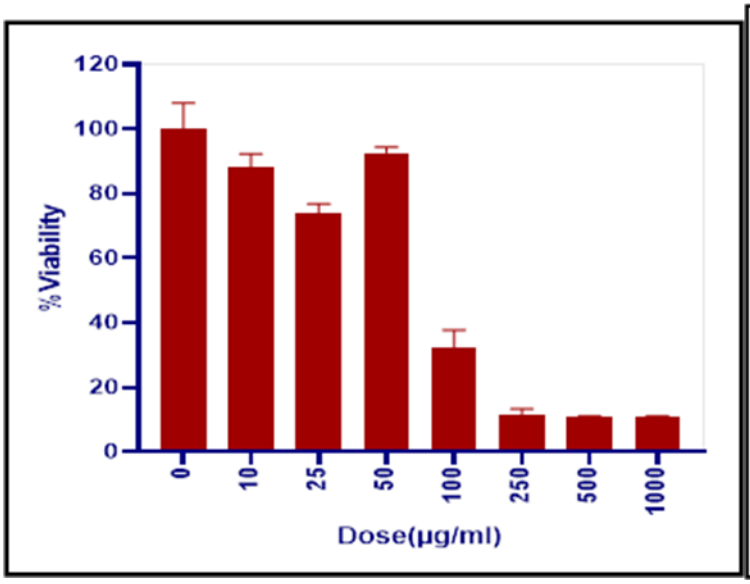

Figure 3. Cell availability of Glyde alone at 48h.

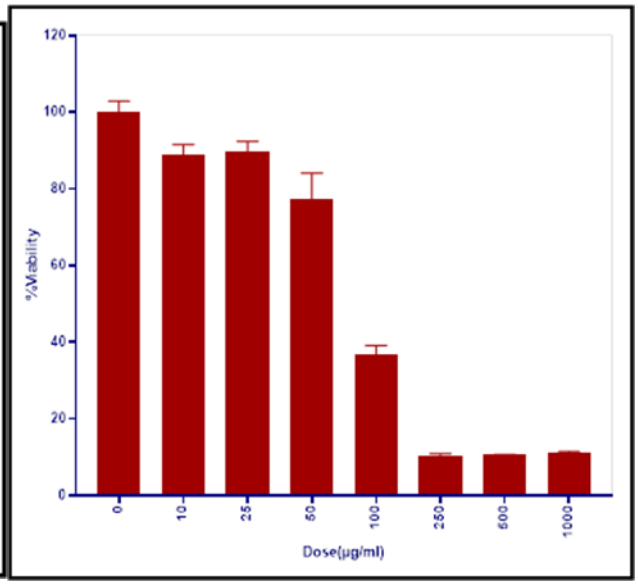

Figure 4. Cell availability of Glyde-Zinc mix at 48h.

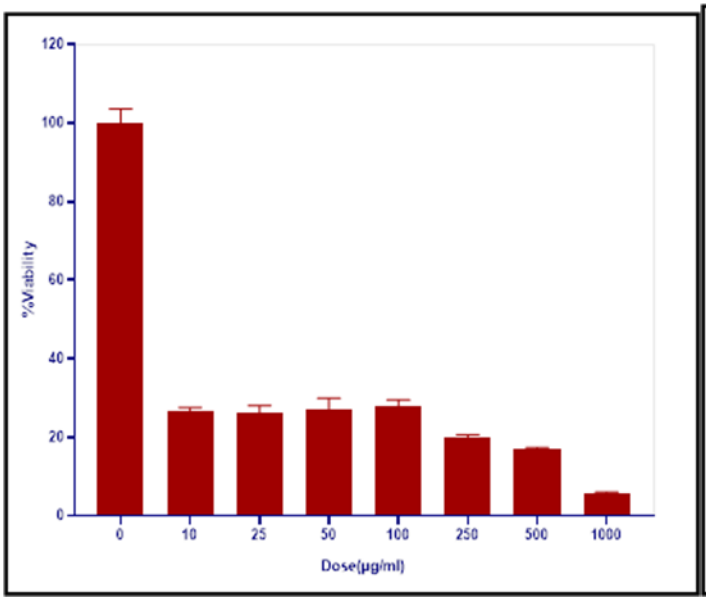

Figure 5. Cell availability of Glyde alone at 72h.

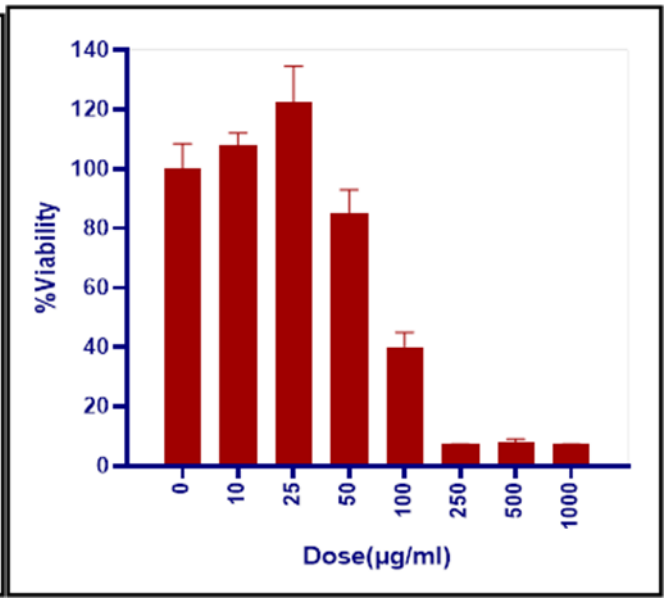

Figure 6. Cell availability of Glyde-Zinc mix at 72h.

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	Glyde only	Plus 3% zinc	Plus 4% zinc	Plus 5% zinc
Min in UV	0.106630904 At 364 nm.	0.295807 At 358 nm.	0.177871 At 380 nm.	0.052597 at 372 nm
Max in UV	4.3346711 At 202 nm.	2.200893 At 192 nm.	4.835236 At 224 nm.	4.492694 At 208 nm.
Min in visible	0.106996147 At 390 nm.	0.296713 At 424 nm.	0.165798 At 602 nm.	0.0453 at 426 nm,
Max in visible	0.131435706 At 780 nm.	0.309136 At 772 nm.	0.178722 At 404 nm.	0.0528 at 382 nm.

Table 4. Minimum and maximum values of wavelength absorption according to Glyde and its additive mixtures.