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Original Research Article

Comparative analysis of antimicrobial efficacy and cytotoxicity of *Terminalia arjuna* bark extract mediated zinc oxide nanoparticles (ZnONPs) and selenium nanoparticles (SeNPs): An in-vitro study

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ABSTRACT

Background: This study provides an in-depth comparative analysis of the antimicrobial efficacy and cytotoxic effects of *Terminalia arjuna*-mediated Selenium Nanoparticles (TA-SeNPs) and Zinc Oxide Nanoparticles (TA-ZnONPs) in the context of chronic periodontitis. The well diffusion assay and Brine Shrimp Lethality Assay were employed to assess antibacterial efficacy and cytotoxicity, respectively.
Aim & Objective: The aim of the study is to contribute to the understanding of the potential applications and safety considerations associated with green-synthesized ZnONPs and SeNPs as a local drug delivery agent in the treatment of chronic periodontitis.The objective of the study is to comparatively analyse the antimicrobial efficacy and cytotoxic effects of *Terminalia arjuna*-mediated Selenium Nanoparticles (TA-SeNPs) and Zinc Oxide Nanoparticles (TA-ZnONPs).
Materials and Methods: Mueller Hinton agar was prepared for the well diffusion assay, and 24-hour-old cultures of *S. mutans*, *E. coli*, and *P. aeruginosa* were swabbed onto the agar. Different concentrations (25, 50, 100 μg/mL) of TA-SeNPs and TA-ZnONPs were loaded into wells, and a plant extract served as the control. Plates were incubated at 37°C for 24 hours, and inhibition zones were measured. For the Brine Shrimp Lethality Assay, a saline solution was prepared, and TA-SeNPs and TA-ZnONPs were introduced at concentrations of 5, 10, 20, 40, and 80μg/mL Viability was observed after a 24-hour incubation.
Results: Employing antimicrobial activity against oral pathogens, SeNPs demonstrated a specific and heightened response against *Streptococcus mutans*, while ZnONPs exhibited consistent efficacy against all tested pathogens, suggesting broad-spectrum potential. However, the Brine Shrimp Lethality Assay revealed SeNPs concentration-dependent cytotoxicity, necessitating careful dosage considerations. In contrast, ZnONPs displayed a more favorable cytotoxic profile.
Conclusion: These small differences underscore the importance of optimizing dosages to balance antimicrobial efficacy and safety, providing valuable details for the development of targeted nanotherapeutics for chronic periodontitis. Further mechanistic studies and in vivo investigations are needed to refine their potential applications in periodontal infection management.

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1. Introduction

Local drug delivery (LDD) is a strategy employed in the management of chronic periodontitis, involving the direct application of antimicrobial treatments into periodontal pockets. This targeted approach aims to eliminate or regulate pathogens, a crucial aspect of periodontal disease therapy. Traditionally, mechanical methods like scaling and root planing (SRP) have been utilized for this purpose, but they can be time-consuming and sometimes ineffective. In contrast, LDD provides a focused and potentially more efficient alternative. Local administration of anti-infective pharmacological agents, particularly antibiotics, is a hallmark of LDD. The doses administered locally are generally much lower than those required for systemic administration, mitigating the risk of serious systemic side effects associated with the drug. Studies have demonstrated that LDD not only offers additional benefits in reducing pocket depth compared to SRP alone but is also well-tolerated by patients. The use of LDD systems in periodontal therapy has undergone extensive research, yielding promising results in the effective control of chronic periodontitis.[1–3]

Local drug delivery systems utilizing SeNPs and ZnO NPs exhibit promise in the management of chronic periodontitis. These nanoparticles, endowed with antimicrobial and anti-inflammatory properties, emerge as suitable candidates for effective periodontal therapy.[4–7] The robust antimicrobial activity of selenium nanoparticles against periodontal pathogens is noteworthy. Moreover, SeNPs have demonstrated the capacity to promote osteoblast differentiation, indicating a dual role in inhibiting bacterial growth and fostering bone regeneration.[3,4] In the case of zinc oxide nanoparticles, their potential application in photoactivated disinfection (PAD) alongside periodontal adhesives has been explored, presenting a promising avenue for the treatment of chronic periodontitis.[4] A shared advantage of both SeNPs and ZnONPs lies in their ability to deliver lower drug doses directly to affected sites. This targeted approach minimizes systemic side effects, contributing to enhanced treatment outcomes.[5,7]

Terminalia arjuna, a medicinal plant, has been extensively studied, revealing its efficacy in combating chronic periodontitis. Aqueous and ethanolic extracts from *Terminalia arjuna* have exhibited significant antimicrobial activity against periodontal pathogens, including *Staphylococcus aureus, Pseudomonas species*, and *Escherichia coli*, along with anti-inflammatory properties.[8–10] The active constituents identified in *Terminalia arjuna* extracts, such as tannins, arjunic acid, arjunogenin, arjunetine, and arjunolone, contribute to their antimicrobial effects.[9,11] Moreover, innovative research has focused on the green synthesis of TA-SeNPs and TA-ZnONPs exploring their antioxidant, antibacterial, and anticancer activities. These nanoparticles, when incorporated into a gel, demonstrated biocompatibility and exhibited potential for diverse biological applications.[10,12]

In this current investigation, *Terminalia arjuna* bark extract was utilized for the synthesis of zinc oxide nanoparticles and selenium nanoparticles. The study aimed to comparatively evaluate the antibacterial activity of these nanoparticles in inhibiting the growth of oral pathogens associated with periodontal diseases using agar well diffusion technique. Additionally, the cytotoxic effects of the synthesized nanoparticles were assessed through the brine shrimp lethality assay, considering their potential application in periodontitis management.

2. Materials and Methods

2.1. Preparation of Terminalia arjuna bark extract

To prepare the *Terminalia arjuna* bark extract, the following steps were performed. 3g of powdered *Terminalia arjuna* was accurately measured and added to 100 mL of distilled water in a beaker. The mixture was stirred well and heated using a heating mantle at a temperature of 60-70 ^{o}C for 20 minutes. After heating, the beaker was removed from the heating mantle and the mixture was allowed to cool down to room temperature. The cooled mixture was filtered using a Whatmann No:1 filter paper to remove any solid impurities. The filtered extract was then stored in a clean and sterile container for later use in the synthesis of selenium and zinc oxide nanoparticles.

2.2. Green synthesis of ZnONPs

In this study, 20mM zinc nitrate was used as a precursor for synthesizing ZnONPs. The precursor was dissolved in 50 mL of distilled water in a conical flask. Then, 50mL of *Terminalia arjuna* bark extract, which was previously filtered, was added to the conical flask. The mixture was stirred at a rate of 700 rpm using a magnetic stirrer for 48 hours. The reaction mixture was allowed to proceed until the zinc ions were reduced and capped by the components in the *Terminalia arjuna* bark extract.

After 48 hours of stirring, the synthesized ZnONPs was centrifuged at 8000 rpm for 10 minutes. This step was necessary to separate the nanoparticle pellet from the supernatant. The pellet containing the synthesized ZnONPs was collected and stored in an airtight Eppendorf tube. The stored sample was used for further characterization and biomedical activities.

2.3. Green synthesis of SeNPs

The synthesis process employed 20mM sodium selenite as the precursor. The precise measurement of 20mM sodium

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selenite was combined with 50mL of distilled water in a conical flask. Subsequently, 50mL of the previously filtered *Terminalia arjuna* extract was introduced into the flask. The reaction mixture underwent continuous stirring on a magnetic stirrer at 700 rpm for an extended duration of 48 hours to facilitate the synthesis of SeNPs. Following synthesis, the SeNP solution underwent centrifugation at 8000 rpm for 10 minutes, effectively separating the pellet from the supernatant. The collected SeNP pellet was carefully stored in an airtight Eppendorff tube.

2.4. Antimicrobial activity

The antimicrobial efficacy of ZnONPs and SeNPs derived from *Terminalia arjuna* was assessed against oral pathogens including *S. mutans, E. coli*, and *Pseudomonas sp*. Various concentrations—namely 25μl, 50μl, and 100μl—were examined using the agar well diffusion method. To initiate the procedure, Mueller Hinton agar was prepared and subjected to sterilization at 120 lbs for a duration of 45 minutes. Subsequent to the sterilization process, the medium was poured into sanitized plates and allowed to solidify.

Wells were generated using a sterile 9mm polystyrene tip. These wells were then loaded with TA-ZnONPs and TA-SeNPs across different concentrations. The chosen test organisms were subsequently swabbed onto the plates. The plates were then incubated at a temperature of 37°C for a period of 24 hours. Amoxicillin was utilized as the standard antibiotic. Following the incubation period, zone of inhibition was measured.[13]

2.5. Cytotoxic effect

A quantity of 2 grams of iodine-free salt was measured and dissolved within 200 mL of distilled water. Following this, 6 well ELISA plates were utilized, and each well was filled with approximately 10 to 12 mL of saline water. Subsequently, 10 nauplii were introduced gradually into every individual well, each containing varying concentrations (5μg, 10μg, 20μg, 40μg, 80μg) of the green synthesized ZnONPs and SeNPs. The plates were then placed under incubation for a duration of 24 hours. Once the 24-hour incubation period had elapsed, the ELISA plates were examined and the count of live nauplii was recorded.[14] The calculation was performed using the subsequent formula: Number of dead nauplii / [number of dead nauplii + number of live nauplii] × 100.

3. Result

3.1. Antibacterial activity

In this study, the antibacterial activities of TA-SeNPs and TA-ZnONPs were systematically evaluated against common oral pathogens, namely *Streptococcus mutans (S. mutans)*, *Escherichia coli* (E. coli), and *Pseudomonas sp*

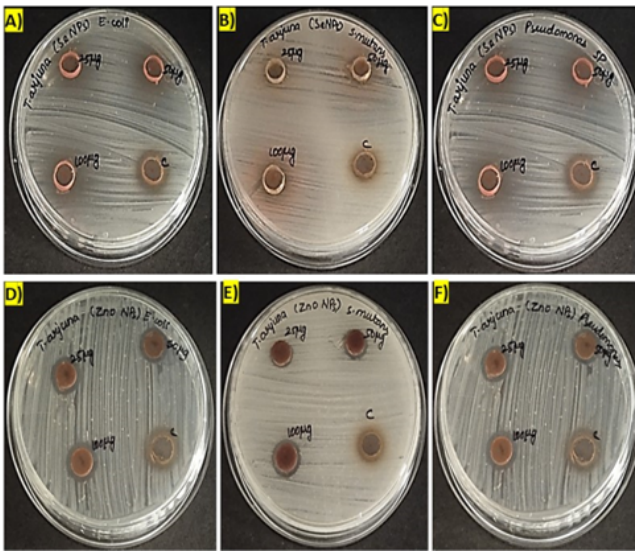

Figure 1: Antibacterial activity ofTA-SeNPs and TA-ZnONPs using agar well diffusion technique**; A):** SeNPs against E.coli**; B):** SeNPs against S.mutans; **C):** SeNPs against Pseudomonas sp**; D):** ZnONPs against E.coli**; E):** ZnONPs against S.mutans; **F):** ZnONPs against Pseudomonas sp

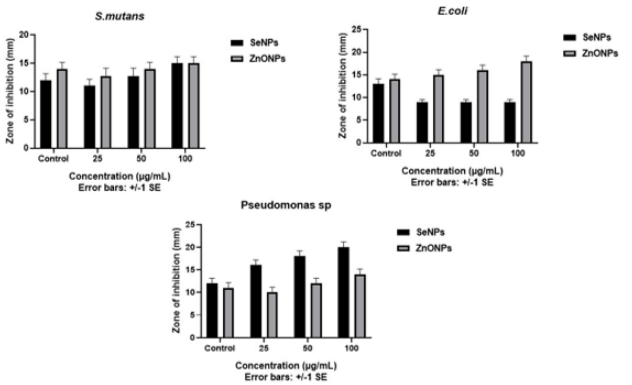

Figure 2: Antibacterial activity of green synthesized SeNPs and ZnONPs against *S.mutans, E.coli, Pseudomonas sp*

(Figure 1).

The investigation involved concentrations of 25μg/mL, 50μg/mL, and 100μg/mL, with a corresponding control group, to discern concentration-dependent responses. SeNPs exhibited a clear dose-dependent antibacterial effect against *S. mutans*, as evidenced by an incremental increase in the zone of inhibition from 11mm at 25μg/mL to 15mm at 100μg/mL, indicative of a heightened antibacterial response with elevated concentrations. In contrast, *E. coli* displayed a consistent zone of inhibition of 9mm across all concentrations, suggesting a potential saturation point beyond which further increases in SeNP concentration may not significantly enhance antibacterial activity against this strain. *Pseudomonas sp* exhibited a

notable concentration-dependent augmentation in the zone of inhibition, progressing from 16mm at 25μg/mL to 20mm at 100μg/mL, underscoring the efficacy of SeNPs against this particular strain (Figure 2).

Shifting the focus to ZnONPs, similar concentration-dependent responses were observed against all three pathogens. For *S. mutans*, the zone of inhibition increased with concentrations, measuring 13mm, 14mm, and 15mm at 25μg/mL, 50μg/mL, and 100μg/mL, respectively. Correspondingly, *Pseudomonas sp* displayed concentration-dependent inhibition zones of 10mm, 12mm, and 14mm at the same respective concentrations. In the case of *E. coli,* the zone of inhibition exhibited a progressive increase from 15mm at 25μg/mL to 18mm at 100μg/mL. Notably, the standard control (*T.arjuna extract*), serving as a comparative benchmark, yielded results comparable to the highest nanoparticle concentration, providing additional validation for the antimicrobial potential of ZnONPs. Overall, the distinct antibacterial responses of TA-SeNPs and TA-ZnONPs against common oral pathogens, as evidenced by their concentration-dependent effects, offer promising avenues for exploring their potential applications in addressing periodontitis, a prevalent oral health concern.

3.2. *Cytotoxic effect*

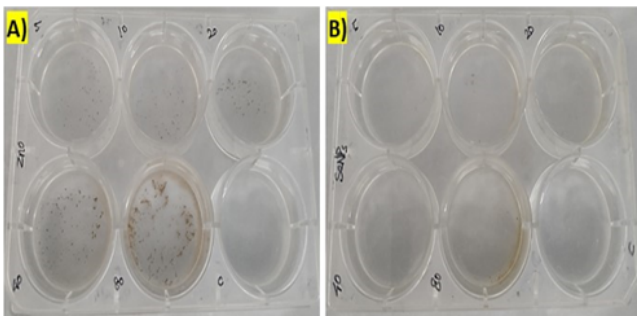

Figure 3: Brine shrimp lethality assay using six well ELISA plate; **A):** ZnONPs; **B):** SeNPs

This comprehensive comparative analysis delves into the distinct cytotoxic effects exhibited by TA-SeNPs and TA-ZnONPs on brine shrimp nauplii through the brine shrimp lethality assay, elucidating small variations at each concentration over a two-day experimental period (Figures 3 and 4).

At 5μg/mL, both SeNPs and ZnONPs manifested 100% viability on Day 1. However, on Day 2, SeNPs displayed an 80% viability, while ZnONPs maintained 100%, suggesting a potential disparity in cytotoxic impact, with ZnONPs exhibiting a lesser effect at this concentration. Advancing to 10μg/mL, SeNPs exhibited 100% viability on Day 1, diminishing to 70% on Day 2, indicating a dose-dependent effect. In parallel, ZnONPs sustained 100% viability on Day 1 but demonstrated a slightly higher viability of 80% on Day 2, implying a potentially milder cytotoxic effect compared to SeNPs.

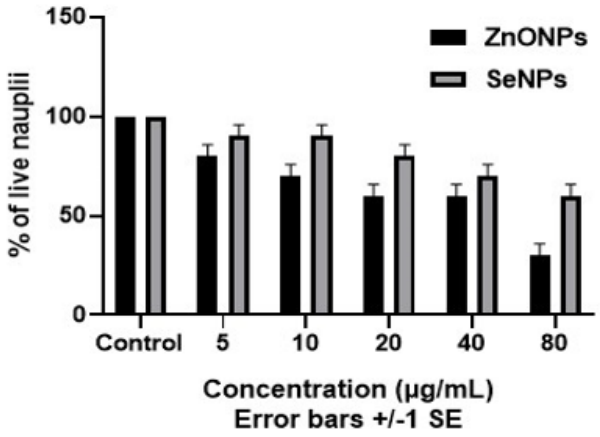

Figure 4: Cytotoxic effect of green synthesized SeNPs and ZnONPs using Brine shrimp lethality assay

At 20μg/mL, both nanoparticles initially secured 100% viability on Day 1. However, SeNPs showed a decline to 60% on Day 2, while ZnONPs maintained a higher viability of 80%, emphasizing a potentially lower cytotoxicity of ZnONPs at this concentration over the experimental period. For 40μg/mL, SeNPs exhibited sustained 60% viability on both days, whereas ZnONPs maintained 100% viability on Day 1 but decreased to 60% on Day 2. This suggests a concentration-dependent impact for both nanoparticles, with SeNPs displaying consistent cytotoxicity and ZnONPs showing a delayed effect. At the highest concentration of 80μg/mL, SeNPs exhibited full viability on Day 1 but a substantial decrease to 30% on Day 2, indicating a pronounced and concentration-dependent lethality. In contrast, ZnONPs demonstrated a higher viability of 60% on Day 2, suggesting a potentially less severe impact on brine shrimp lethality at this concentration. Overall, this detailed comparative analysis of the cytotoxic effects of SeNPs and ZnONPs on brine shrimp nauplii, considering variations at each concentration, offers critical insights. These findings contribute to a comprehensive understanding of the potential biological impact of nanoparticles, a crucial consideration for their safe and effective utilization in various applications, including those related to periodontitis and oral health.

4. Discussion

The assessment of the antimicrobial activity of TA-SeNPs and TA-ZnONPs against common oral pathogens, including *Streptococcus mutans (S. mutans)*, *Escherichia coli* (E. coli), and *Pseudomonas sp.*, is pivotal in understanding their potential efficacy in addressing periodontitis, a prevalent oral health concern.

Selenium nanoparticles (SeNPs) have demonstrated notable antibacterial properties against periodontal pathogens. In an investigation conducted by Hou et al., it was observed that SeNPs, when present at concentrations of 64 μg/ml and below, not only facilitated osteoblastic differentiation but also hindered the proliferation of *Porphyromonas gingivalis*, a recognized periodontal pathogen.[14] Barma and Doraikanan et al., further explored the antimicrobial capabilities of SeNPs by utilizing *Clitoriaternatea* flower extract for synthesis. Their study highlighted the potential of these SeNPs in combating gram-positive bacteria, specifically *Staphylococcus aureus*.[15] Collectively, these findings underscore the promising prospects of SeNPs as effective antimicrobial agents against periodontal pathogens.

Similar concentration-dependent responses were observed for ZnONPs against all three pathogens. The progressive increase in the zone of inhibition for *S. mutans, E. coli,* and *Pseudomonas sp* at higher concentrations (25μg/mL, 50μg/mL, and 100μg/mL) demonstrates the efficacy of ZnONPs in inhibiting the growth of these oral pathogens. The comparable results of the standard control (*Terminalia arjuna* extract) to the highest nanoparticle concentration further validate the antimicrobial potential of ZnONPs. This alignment suggests that the antimicrobial properties observed are attributed to the synthesized ZnONPs rather than residual components from the *Terminalia arjuna* extract. The concentration-dependent antibacterial responses of both SeNPs and ZnONPs underscore their potential applications in addressing periodontitis. Periodontitis involves a complex interplay of different bacterial species, and the ability of these nanoparticles to exhibit varying responses against different pathogens is promising.[16]

On comparing the antibacterial activity of SeNPs and ZnONPs, both exhibit concentration-dependent responses, but SeNPs demonstrate a more pronounced effect against *S. mutans*, whereas ZnONPs show consistent efficacy against all tested pathogens. The strain-specific response of SeNPs and the broad-spectrum activity of ZnONPs present interesting details in their potential application against the diverse microbial population associated with periodontitis. ZnONPs have displayed promising antibacterial properties against periodontal pathogens. In a particular investigation, ZnONPs were synthesized and assessed for their efficacy against *Streptococcus spp*., the bacteria associated with dental caries. The findings indicated that ZnONPs exhibited anti-*Streptococcus* activity, particularly at a concentration of 64 μg/ml.[17] Another research focus centered on biogenic ZnONPs derived from *Andrographis paniculata* leaves aqueous extract (APLAE). These biogenic ZnONPs demonstrated potent inhibitory activity against bacteria linked to periimplantitis, including *Escherichia coli* and *Staphylococcus aureus*.[18] Additionally, a series of nanoparticles, ZnO among them, underwent testing against oral pathogens such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. The outcomes underscored the significant inhibitory effect of ZnONPs on these pathogens, with SiO_2 and $CaCO_3$ nanoparticles exhibiting noteworthy effectiveness, respectively.[19] Collectively, these studies suggest that ZnONPs possess antibacterial potential against periodontal pathogens and hold promise for application in dental contexts.

The brine shrimp lethality assay provides insights into the potential cytotoxic effects of SeNPs and ZnONPs, a critical aspect for evaluating their safety in biomedical applications. The concentration-dependent decrease in viability of brine shrimp nauplii suggests a dose-dependent cytotoxic effect of SeNPs. The notable decrease from 100% viability on Day 1 to 80% on Day 2 at 5μg/mL, and the substantial decrease to 30% at the highest concentration of 80μg/mL, indicates a pronounced and concentration-dependent lethality. This observation raises considerations for the optimal dosage of SeNPs to balance antimicrobial efficacy with potential cytotoxic effects. Previous studies have indicated that SeNPs exhibit minimal cytotoxic effects against Artemia salina, implying their potential biocompatibility.[20] Furthermore, the brine shrimp assay was utilized to assess SeNPs enhanced with clove and cardamom extract.[21] These results affirm the potential applicability of SeNPs across diverse industries, such as food, biomedical, and cosmetics, owing to their low cytotoxicity and biocompatible characteristics.[22]

In contrast, ZnONPs exhibit a more favorable cytotoxic profile. Maintaining 100% viability at lower concentrations (5μg/mL and 10μg/mL) on Day 2 and a gradual decrease to 60% at the highest concentration of 80μg/mL suggests a potentially lower cytotoxic impact compared to SeNPs. This differential cytotoxic response indicates the importance of considering both nanoparticle type and concentration in biomedical applications. The cytotoxic effects observed in the brine shrimp lethality assay provide crucial information for understanding the potential biological impacts of SeNPs and ZnONPs. While their antibacterial properties make them promising candidates for periodontitis treatment, the concentration-dependent cytotoxicity highlights the need for careful dosage considerations to ensure safety. Multiple investigations have utilized the brine shrimp lethality assay to assess the cytotoxicity of zinc oxide nanoparticles (ZnONPs). Additionally, a comparative analysis of the impact of Se and ZnO NPs on brine shrimp larvae revealed that the toxicity of these nanoparticles was contingent on their size and solubility.[13] These findings collectively suggest that ZnONPs can exert cytotoxic effects on brine shrimp larvae, and various factors such as concentration, size, and solubility may influence their toxicity.

On comparing the cytotoxic effects of SeNPs and ZnONPs, SeNPs exhibit a more significant decrease

in viability at higher concentrations, emphasizing the importance of dose optimization. ZnONPs, on the other hand, maintain higher viability even at elevated concentrations, suggesting a potentially safer profile in terms of cytotoxicity.

Overall, both SeNPs and ZnONPs show promising antimicrobial activity against oral pathogens relevant to periodontitis. However, their cytotoxic effects exhibit distinct patterns, with SeNPs demonstrating a more pronounced decrease in viability at higher concentrations compared to ZnONPs. These small variations emphasize the importance of a careful balance between antimicrobial efficacy and safety considerations, particularly in the context of developing therapeutic interventions for periodontitis.

The multifaceted biological properties of TA-SeNPs and ZnONPs suggest that *Terminalia arjuna* holds promise as a local drug delivery agent for the treatment of chronic periodontitis. These findings emphasize the potential of *Terminalia arjuna* in the development of effective therapeutic strategies for periodontal health.

5. Conclusion

The comprehensive investigation into the antimicrobial and cytotoxic properties of TA-SeNPs and TA-ZnONPs reveals distinctive characteristics with potential implications for chronic periodontitis management. SeNPs exhibit a strain-specific antibacterial response, notably against *Streptococcus mutans*, emphasizing their specificity in targeting pathogens associated with dental caries and periodontitis. Conversely, ZnONPs display consistent efficacy against a spectrum of oral pathogens, suggesting their applicability in addressing the polymicrobial nature of periodontal infections. The concentration-dependent cytotoxic effects observed in SeNPs, as evidenced by the brine shrimp lethality assay, underscore the necessity for careful dosage optimization to balance therapeutic efficacy and safety. ZnONPs, on the other hand, present a more favorable cytotoxic profile. These findings underscore the imperative of tailoring dosage strategies for effective and safe nanotherapeutic interventions against chronic periodontitis.

6. Source of Funding

None.

7. Conflict of Interest

None.

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