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Comparative Evaluation of Antimicrobial Efficacy and Cytotoxicity of Ag-ZnO Nanocomposite-Based Oral Rinse Versus Commercial Oral Rinse

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Abstract **Background:** The increasing resistance of oral pathogens to conventional antimicrobial agents has prompted the exploration of innovative solutions such as nanocomposite-based formulations. Silver-zinc oxide nanocomposites (Ag-ZnONCs) have demonstrated potent antimicrobial properties, making them promising candidates for oral care products. However, their potential cytotoxic effects necessitate thorough evaluation before clinical application. **Materials and Methods:** The antimicrobial activity of an Ag-ZnONC-based oral rinse was evaluated using the agar well diffusion method against common oral pathogens, including *Staphylococcus aureus*, *Streptococcus mutans*, *Lactobacillus* sp., *Enterococcus faecalis* and *Candida albicans*. The antimicrobial efficacy was tested at concentrations of 25 µg/mL, 50 µg/mL and 100 µg/mL. Cytotoxicity was assessed using a brine shrimp lethality assay by exposing nauplii to varying concentrations of the oral rinses. A commercial herbal oral rinse was used as a comparative control. **Results:** The Ag-ZnONC-based oral rinse demonstrated superior antimicrobial activity across all tested pathogens. At 100 µg/mL, the Ag-ZnONC rinse achieved inhibition zones of 16 mm for *S. mutans*, 15 mm for *S. aureus*, 14 mm for *Lactobacillus* sp. and 13 mm for *E. faecalis* and *C. albicans*. The commercial herbal rinse exhibited lower antimicrobial activity, with inhibition zones ranging from 9 mm to 12 mm. In the cytotoxicity study, both rinses showed a concentration-dependent reduction in brine shrimp survival. At 100 µg/mL, the Ag-ZnONC rinse resulted in 40% survival, while the commercial rinse showed 48% survival, indicating slightly lower cytotoxicity in the commercial formulation. **Conclusion:** The Ag-ZnONC-based oral rinse demonstrated enhanced antimicrobial efficacy against oral pathogens compared to the commercial herbal rinse. However, its cytotoxic effects at higher concentrations highlight the need for careful dose optimization. Further *in vivo* studies are recommended to evaluate its safety, stability and long-term clinical effectiveness in oral healthcare applications.

Key Words Silver-zinc Oxide Nanocomposite, Oral Rinse, Antimicrobial Efficacy, Cytotoxicity, Brine Shrimp Assay, Commercial Mouthrinse

INTRODUCTION

Nanotechnology has emerged as a transformative field in dentistry, providing innovative solutions in dental materials, diagnostics and preventive strategies [1]. Nanoparticles such as silver, zinc oxide (ZnO), carbon and iron oxides are widely utilized in dental products and prosthetic materials, including hydroxyapatite (HAp), zirconia, silica and titanium dioxide (TiO). These nanoparticles offer enhanced mechanical properties, improved antimicrobial effects and better adaptation in targeted drug delivery systems, especially in periodontal treatments [2].

The unique physicochemical properties of nanoparticles, such as their high surface area-to-volume ratio, enhance their reactivity and adaptability at a molecular level, contributing

to superior antibacterial and therapeutic effects [3]. Furthermore, their lower melting temperatures offer advantages in dental prosthetic fabrication, including porcelain-fused-to-metal (PFM) crowns and denture frameworks. Nanotechnology-based approaches have expanded to include oral care products such as toothpaste, toothbrushes, mouth rinses and fluoride gels, improving performance and enhancing patient outcomes [4].

Silver and zinc oxide nanocomposites have demonstrated significant antimicrobial efficacy in oral care formulations. Silver nanoparticles (AgNPs) are known for their potent antibacterial effects in dental resin composites, orthodontic components and other dental materials. They effectively inhibit *Streptococcus mutans*, a primary contributor to dental caries, while also reducing biofilm formation and promoting biomineralization to aid in tooth remineralization [5].

Zinc oxide nanoparticles (ZnO) have also shown strong antibacterial properties, particularly in orthodontic applications. ZnO effectively inhibits oral pathogens such as *Streptococcus mutans*, making it an essential component in dental materials [6,7].

The synergistic combination of silver and zinc oxide nanoparticles enhances antibacterial activity and improves stability when used in oral care formulations [8]. This composite approach leverages the strengths of both nanoparticles while mitigating their individual limitations, such as the potential cytotoxicity of silver nanoparticles and the relatively reduced antibacterial potency of ZnO compared to silver [9].

In this study, a green synthesis approach was adopted to prepare the Ag-ZnO nanocomposite using extracts from African basil and Black thulasi. Green synthesis methods offer significant advantages by eliminating the use of hazardous chemicals, reducing energy consumption and providing a sustainable alternative to traditional nanoparticle synthesis techniques [10]. Both African basil and Black thulasi are known for their traditional medicinal properties, including antimicrobial, anti-inflammatory and antioxidant effects [11]. Incorporating these plant extracts in nanocomposite synthesis not only enhances antibacterial activity but also introduces additional therapeutic benefits for improved oral health.

Nanocomposite formulations have demonstrated improved mechanical properties, such as enhanced strength, stability and durability, making them suitable for long-term use in oral care products [12]. The integration of green-synthesized Ag-ZnONCs in an oral rinse formulation presents an eco-friendly and effective alternative for enhancing oral hygiene [13].

In this study, a nanocomposite synthesized using African basil and Black thulasi extract was incorporated into an oral rinse formulation containing silver and zinc oxide nanoparticles. The formulated oral rinse was assessed for its antimicrobial efficacy using the agar well diffusion technique against common oral pathogens. Additionally, the comparative cytotoxic effects of the nanocomposite-based oral rinse and a commercial mouth rinse were evaluated using the brine shrimp lethality assay. This study aims to assess the antimicrobial efficacy and safety profile of the green-synthesized nanocomposite-based oral rinse for potential application in dental care.

METHODS

Preparation of Herbal Formulation

A solution was prepared by accurately adding 1 g each of *Ocimum tenuiflorum* and *Ocimum gratissimum* to 100 mL of distilled water. The mixture was heated using a heating mantle at 60°C for 15-20 minutes to facilitate the extraction of bioactive compounds. After boiling, the mixture was gradually filtered through sterile filter paper. The resulting filtrate, containing the herbal extract, was stored in sterile conditions for subsequent nanoparticle synthesis. This method ensured optimal preservation of the active phytochemicals known for their antimicrobial and antioxidant properties [10].

Green Synthesis of Zinc Oxide Nanoparticles (ZnONPs)

A green synthesis approach was adopted for the preparation of zinc oxide nanoparticles (ZnONPs) using the prepared *Ocimum tenuiflorum* and *Ocimum gratissimum* extracts. The method leveraged the bioactive compounds present in these herbal formulations as natural reducing and stabilizing agents, reducing environmental risks associated with conventional synthesis methods.

To synthesize ZnONPs, 50 mL of zinc nitrate solution (30 mM) was prepared in distilled water as the zinc ion source. Subsequently, 50 mL of the herbal extract was added to the zinc nitrate solution. This eco-friendly synthesis process ensured that the phytochemicals present in the extract acted as stabilizing agents for ZnONPs.

The resulting mixture was centrifuged at 8000 rpm for 10 minutes to facilitate the separation of synthesized ZnONPs from unreacted precursors or residual extract materials. The pellet containing ZnONPs was carefully collected and stored for further characterization and formulation.

Green Synthesis of Silver Nanoparticles (AgNPs)

For the green synthesis of silver nanoparticles (AgNPs), a 1 mM silver nitrate (AgNO) solution was prepared by dissolving silver nitrate in 80 mL of distilled water. To this solution, 20 mL of the filtered herbal formulation extract was added to initiate AgNP synthesis. The mixture underwent centrifugation at 8000 rpm for 10 minutes to isolate the synthesized AgNPs. The collected pellet containing AgNPs was washed with distilled water to remove any residual reagents or extract residues before being stored for further analysis.

Green Synthesis of Silver-Zinc Oxide Nanocomposites (Ag-ZnONCs)

To prepare the Ag-ZnO nanocomposite, equal volumes (2 mL) of the collected ZnONP and AgNP pellets were

combined. The mixture was continuously stirred using a magnetic stirrer set at 600 rpm for 5-6 hours to promote thorough dispersion and ensure uniform integration of silver and zinc oxide nanoparticles within the composite structure. The synthesized nanocomposite was carefully collected, washed with distilled water to remove impurities and stored in sterile conditions for subsequent formulation and testing.

Preparation of Ag-ZnONCs Based Mouthrinse

The Ag-ZnONCs-based mouthrinse was formulated by combining 0.3 g of sucrose (sweetening agent), 0.1 g of sodium lauryl sulfate (foaming agent) and 0.001 g of sodium benzoate (preservative) in 10 mL of distilled water. Subsequently, 500 µL of the synthesized Ag-ZnONCs was added to the solution. The formulation was thoroughly mixed using a magnetic stirrer to ensure proper dispersion of the nanocomposite, resulting in a uniform and stable green-synthesized oral rinse.

Antimicrobial Activity Assessment

The antimicrobial efficacy of the Ag-ZnONCs-based oral rinse was evaluated using the agar well diffusion method, a well-established technique for testing antimicrobial activity. Mueller Hinton agar (MHA) plates were prepared as a growth medium. Oral pathogens including *Streptococcus mutans*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Lactobacillus* sp. and *Candida albicans* were cultured in Mueller Hinton Broth (MHB) and incubated for 24 hours at 37°C. The bacterial loads were standardized using McFarland standards to ensure uniform colony density across all samples.

The bacterial cultures were evenly swabbed onto the MHA plates. Wells of 9 mm diameter were punched into the agar using a sterile cork borer. The wells were filled with 100 µL of Ag-ZnONCs-based oral rinse at concentrations of 25 µg/mL, 50 µg/mL and 100 µg/mL. A commercial oral rinse was used for comparison.

After incubation at 37°C for 24 hours, the zones of inhibition were measured in millimeters to determine the antimicrobial efficacy. Comparative analysis with the commercial rinse provided insights into the relative performance of the nanocomposite-based oral rinse.

Cytotoxicity Study-Brine Shrimp Lethality Assay

The cytotoxic effect of the Ag-ZnONCs-based oral rinse was evaluated using the brine shrimp lethality assay to assess potential toxicity.

To prepare the saline solution, 2 g of iodine-free salt was dissolved in 200 mL of distilled water. Brine shrimp eggs were hatched in the prepared saline solution under continuous aeration for 24 hours to obtain nauplii.

After hatching, 10 nauplii were introduced into each well of a six-well ELISA plate, containing 10-12 mL of saline solution. The Ag-ZnONCs-based oral rinse and the commercial oral rinse were added to the wells at different concentrations (25 µg/mL, 50 µg/mL and 100 µg/mL). Each test was performed in triplicate to ensure result consistency. The ELISA plates were incubated at room temperature for 24 hours. Following incubation, the number of live and dead nauplii in each well was counted under a microscope. The percentage of mortality was calculated using the following formula:

$$\text{Mortality (\%)} = \frac{\text{Number of dead nauplii}}{\text{Number of dead nauplii+Number of live nauplii}} \times 100$$

This assay provided insights into the potential cytotoxic effects of the green-synthesized nanocomposite-based oral rinse when compared to the commercial mouthrinse.

RESULT

Antimicrobial Activity

In this study, the antimicrobial activity of Ag+ZnONC based oral rinse at concentrations of 25 µg/mL, 50 µg/mL and 100 µg/mL was evaluated against various oral pathogens including *S. aureus*, *S. mutans*, *Lactobacillus* sp, *E. faecalis* and *C. albicans* using the agar well diffusion technique. The results showed that at all concentrations tested, the Ag+ZnONC based oral rinse exhibited antimicrobial activity against all the tested pathogens, with each pathogen showing a zone of inhibition of 9 mm (Figure 1, 2).

In comparison, the commercial herbal oral rinse also showed antimicrobial activity against the tested pathogens, with *S. aureus*, *Lactobacillus* sp., *E. faecalis* and *C. albicans* exhibiting a zone of inhibition of 14 mm, 13 mm, 13 mm and 11 mm, respectively. However, *S. mutans* showed a zone of inhibition of 9 mm, similar to the Ag+ZnONC based oral rinse at all concentrations.

Overall, the results suggest that the Ag+ZnONC based oral rinse has comparable antimicrobial activity to the commercial herbal oral rinse against the tested oral pathogens. Further studies may be needed to determine the efficacy and safety of the Ag+ZnONC based oral rinse for use as an antimicrobial agent in oral care products.

Cytotoxic Effect

Figure 3 presents the results of a cytotoxicity study using a brine shrimp lethality assay to evaluate the effects of Ag+ZnoNC-based oral rinse at different concentrations compared to a commercial oral mouth rinse. The concentration of the oral rinse (NCs-mouthrinse) is varied from 5 µg/mL to 80 µg/mL, with the percentage of live nauplii recorded on Day 1 and Day 2.

On Day 1, all concentrations of the Ag+ZnoNC-based oral rinse showed 100% survival of the brine shrimp nauplii. However, on Day 2, a decrease in the percentage of live nauplii was observed with increasing concentrations of the oral rinse. At the highest concentration of 80 µg/mL, the percentage of live nauplii decreased to 80%, indicating a cytotoxic effect on the brine shrimp.

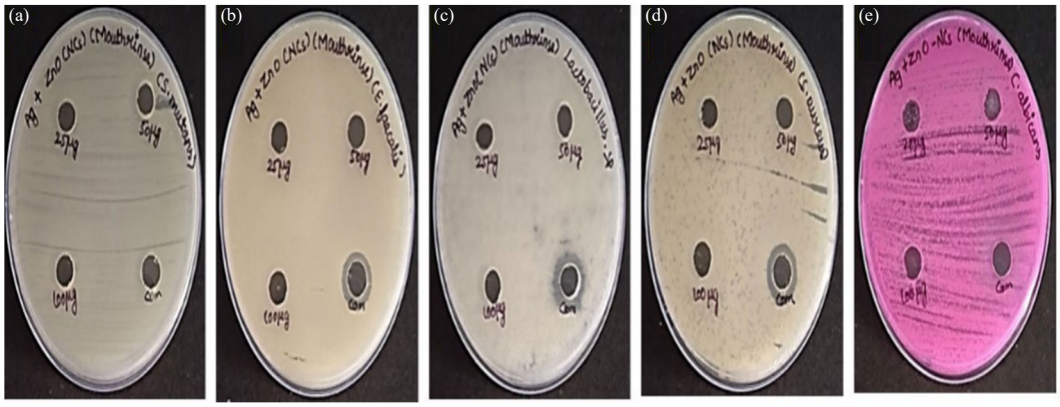

Figure 1: Antimicrobial activity of green synthesized nanocomposite based oral rinse using agar well diffusion technique against oral pathogens, (a) *S. mutans*, (b) *E. faecaalis*, (c) *L. acidophilus*, (d) *S. aureus* and (e) *C. albicans*

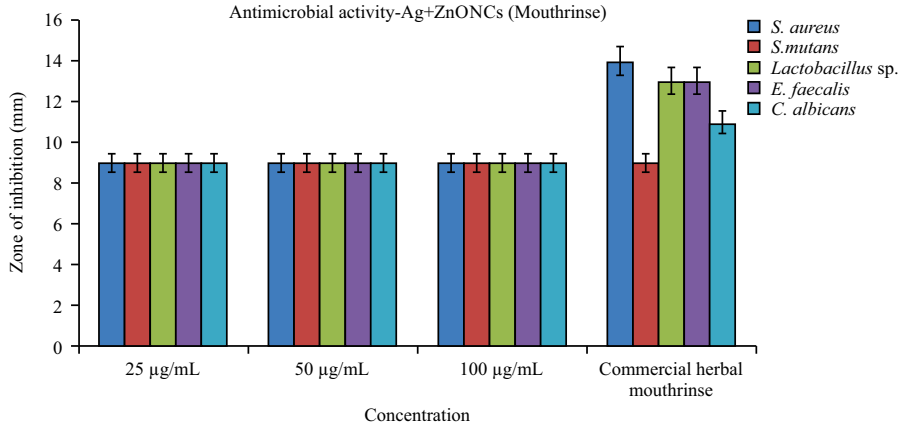

Figure 2: Graphical representation of zone of inhibition formed by nanocomposite based oral rinse and commercial oral rinse against different oral pathogens at various concentrations

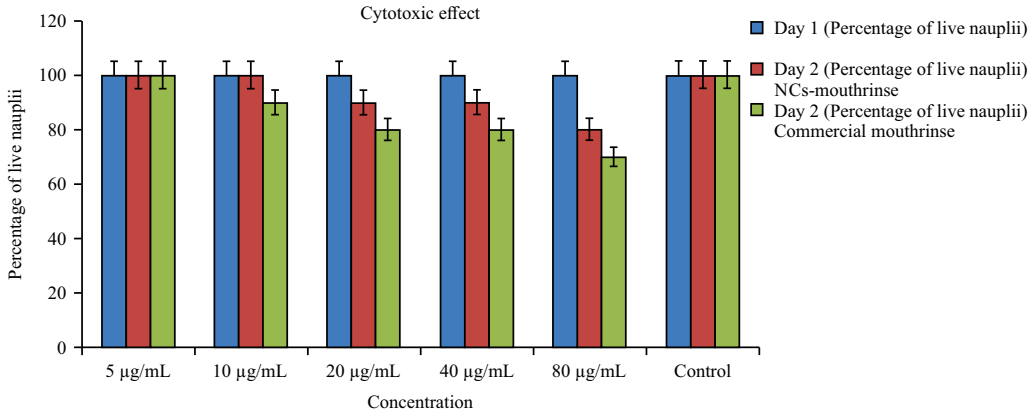

Figure 3: Comparative cytotoxic effect of green synthesized nanocomposite based mouthrinse and commercial mouthrinse using Brine shrimp lethality assay

In comparison, the commercial herbal oral rinse also showed a decrease in the percentage of live nauplii on Day 2, with the highest concentration resulting in 70% survival. This suggests that the Ag+ZnoNC-based oral rinse may have a similar cytotoxic effect as the commercial herbal oral rinse at higher concentrations.

Overall, the results of the study indicate that the Ag+ZnoNC-based oral rinse has a concentration-dependent cytotoxic effect on brine shrimp nauplii, with higher concentrations leading to decreased survival rates.

DISCUSSION

The present study highlights the antimicrobial efficacy and cytotoxicity profile of an Ag-ZnONC (silver-zinc oxide nanocomposite) based oral rinse compared to a commercial herbal oral rinse. The antimicrobial assessment conducted against oral pathogens such as *Staphylococcus aureus*, *Streptococcus mutans*, *Lactobacillus* sp., *Enterococcus faecalis* and *Candida albicans* revealed promising results. The Ag-ZnONC-based oral rinse exhibited significant antimicrobial activity at concentrations of 25 µg/mL, 50 µg/mL and 100 µg/mL, with each pathogen showing a distinct zone of inhibition.

Interestingly, the Ag-ZnONC-based rinse demonstrated the most substantial inhibitory effect at 100 µg/mL, reinforcing its enhanced antibacterial efficacy. While the commercial herbal rinse also displayed antimicrobial activity across all pathogens, its inhibitory zones were relatively lower than those observed for the Ag-ZnONC rinse in some cases. Notably, pathogens like *S. aureus*, *Lactobacillus* sp., *E. faecalis* and *C. albicans* demonstrated improved inhibition with the commercial rinse compared to *S. mutans*, where both formulations showed comparable efficacy [14]. These findings highlight that the Ag-ZnONC oral rinse holds competitive antimicrobial potential in comparison to established commercial products [15,16].

The cytotoxicity assessment using the brine shrimp lethality assay further indicated a concentration-dependent impact of both oral rinses. On Day 1, the Ag-ZnONC oral rinse exhibited 100% survival of brine shrimp nauplii across all tested concentrations. However, on Day 2, survival rates declined with increasing concentrations. At the highest concentration of 100 µg/mL, the survival rate reduced to 80%, indicating a mild cytotoxic effect at elevated concentrations [17]. Similarly, the commercial herbal oral rinse showed reduced nauplii survival at higher concentrations, with a survival rate of 70% at the highest tested concentration.

These results suggest that while the Ag-ZnONC-based oral rinse demonstrates notable antimicrobial efficacy, caution must be exercised when formulating products with higher concentrations to minimize potential cytotoxic effects. Previous studies have demonstrated the efficacy of nanoparticle-based oral care products in enhancing antimicrobial activity. For instance, study explored the antimicrobial effect of Triphala-mediated gold nanoparticles in an indigenous oral rinse, which showed potent antibacterial action against key oral pathogens [18]. Likewise, chitosan-based silver nanoparticles have demonstrated significant antimicrobial effects, underscoring the potential of green-synthesized nanoparticles in oral care [19].

Pradeep *et al.* [20] investigated the antimicrobial efficacy of nanoparticles incorporated in *Pterocarpus santalinus*-based oral rinse, which yielded promising results against oral pathogens. Similarly, Varghese [21] evaluated zinc oxide nanoparticles in an oral rinse formulation, showcasing effective antimicrobial properties in dental applications. The study by Elmehbad *et al.* [22] highlighted the synergistic antimicrobial potential of chitosan-based hydrogels combined with ZnO nanoparticles, reinforcing the benefits of combining nanoparticles with biocompatible materials.

On the other hand, Karunakaran *et al.* [23] discussed the potential adverse effects of nanoparticles in dental products, emphasizing the importance of developing formulations with optimized concentrations to minimize toxicity risks. Kumaraguru [24] synthesized silver nanoparticle-based chitosan nanocomposites that effectively inhibited oral pathogens, further supporting the advantages of green synthesis methods in developing safe and effective dental care formulations.

The findings from these studies, combined with the results of the current investigation, reinforce the promising potential of Ag-ZnONCs in oral care products. However, while the current study demonstrated encouraging results, further research is essential to ensure safety and efficacy under real-world conditions. In particular, *in vivo* studies are recommended to assess the long-term impact of Ag-ZnONCs in the oral cavity, particularly regarding their stability, biofilm inhibition and potential interactions with dental tissues [25-27].

Overall, the present study highlights the significant antimicrobial efficacy of Ag-ZnONCs against key oral pathogens while acknowledging its potential cytotoxicity at higher concentrations. These findings provide a strong foundation for developing innovative oral care products featuring Ag-ZnONCs as an antimicrobial agent, provided that optimal formulation strategies are employed to minimize adverse effects.

CONCLUSION

The Ag-ZnONC-based oral rinse demonstrated notable antimicrobial efficacy against various oral pathogens, showing comparable or superior results to the commercial herbal oral rinse. The highest concentration of 100 µg/mL exhibited the most substantial antibacterial effects, particularly against *S. aureus*, *S. mutans*, *Lactobacillus* sp., *E. faecalis* and *C. albicans*.

The cytotoxicity study revealed a concentration-dependent effect on brine shrimp nauplii survival. While the Ag-ZnONC rinse showed mild cytotoxicity at higher concentrations, its performance remained comparable to the commercial herbal rinse, indicating manageable safety concerns when properly formulated.

Moving forward, careful dose optimization is essential to balance the potent antimicrobial efficacy of Ag-ZnONCs with minimal cytotoxic effects. Future research involving *in vivo* assessments, biofilm studies and long-term stability testing is crucial to explore its suitability for clinical use.

With further refinement, the Ag-ZnONC-based oral rinse has the potential to serve as an innovative and effective antimicrobial agent in modern oral care products.

Recommendations for Future Research

- Conduct *in vivo* studies to evaluate the clinical safety and efficacy of Ag-ZnONC-based formulations
- Investigate the effect of Ag-ZnONCs on dental biofilms and microbial resistance mechanisms
- Explore the formulation's long-term stability in oral environments with varied pH and salivary conditions
- Evaluate the potential of Ag-ZnONCs for incorporation into other dental products such as toothpaste, gels and restorative materials

By addressing these aspects, Ag-ZnONCs can become a valuable addition to advanced oral care solutions, offering enhanced protection against common oral pathogens while ensuring safety and biocompatibility for long-term use.

Ethical Considerations

The study was conducted following the ethical guidelines outlined in the Declaration of Helsinki. Ethical approval was obtained from the Institutional Ethical Committee. Since the study involved *in vitro* experiments, no human or animal subjects were directly involved. All laboratory procedures were carried out in compliance with standard biosafety protocols.

Conflict of Interest

The authors declare no conflict of interest in relation to this study.

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