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Dentistry Section

Comparative Evaluation of Cytotoxicity of Silver and Zinc Oxide Nanoparticles-based Herbal Oral Rinse and Commercially Available Oral Rinse: An In-vitro Study

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ABSTRACT

Introduction: Oral hygiene is a cornerstone of overall health and its importance in dentistry cannot be overstated. Maintaining good oral hygiene is essential not just for preventing tooth decay and gum disease but also for improving general health and well-being. Commercial oral rinses can be a useful addition to an oral hygiene routine, but they are not a cure-all and should be used with caution. Although many commercial oral rinses are available in the market, the therapeutic effects of these products are questionable. In the present study, a unique oral rinse formulation incorporating African basil and black tulsi herbal extracts, combined with silver and zinc oxide nanocomposites (Ncs), was developed and assessed for its cytotoxic properties using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay, with the aim of determine if the herbal oral rinse served as a safe and effective alternative to commercial oral rinses commonly used for oral hygiene.

Aim: To compare the cytotoxic effects of silver nanocomposite-based oral rinse and commercial oral rinse on mouse fibroblast cell viability using the MTT assay across a range of concentrations.

Materials and Methods: The present in-vitro study was conducted in the research laboratory of Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India, over a period of six months starting from March 2024 and concluding on August 2024. In the present study, the green synthesis of Zinc Oxide Nanoparticles (ZnONPs) and Silver and Zinc Oxide Nanoparticles (AgNPs) was carried out utilising African basil and black tulsi extracts (Ocimum tenuiflorum and Ocimum gratissimum) in the presence of a zinc nitrate solution (30 mM in 50 mL distilled water) and a 1 mM silver nitrate solution, respectively. Following the preparation of 100 mL of nanocomposite-based herbal oral rinse, mouse fibroblast cells were exposed to varying concentrations of nanocomposite-based oral rinse and commercial oral rinse (Listerine oral rinse) and cell viability was assessed using the MTT assay. The test was repeated five times at each concentration and the cytotoxic effects of the oral rinses were compared. The Mann-Whitney U test was used to compare the mean values between the two study groups {commercial oral rinse (group-1), nanocomposite oral rinse (group-2)} and $p<0.05$ was considered statistically significant.

Results: The commercial oral rinse used, Listerine (Listerine Oral Rinse-Johnson and Johnson Ltd., Kolhapur, India, Batch No: MK0068), exhibited a dose-dependent cytotoxic effect, with decreasing cell viability percentages as concentrations increased: 80% at 5 µg/mL down to 30% at 100 µg/mL. In comparison, the nanocomposite-based oral rinse also showed reduced cell viability with increasing concentrations but to a lesser extent: from 85% at 5 µg/mL to 35% at 100 µg/mL. The differences in cytotoxicity between the two oral rinses were evident across all concentrations tested, suggesting a potentially milder impact of the nanocomposite-based oral rinse on cell viability compared to commercial oral rinse. Silver nanocomposite-based oral rinse consistently maintained higher cell viability percentages compared to the commercial oral rinse across all tested concentrations, indicating a potentially milder cytotoxic impact on fibroblast cells.

Conclusion: The study demonstrates that nanocomposite-based oral rinse has a less cytotoxic impact on mouse fibroblast cells compared to commercial oral rinse. These results emphasise the potential benefits of nanocomposite-based formulations in oral care products for maintaining optimal cell viability.

Keywords: Biocompatible, Cell viability, Commercial oral rinse, Nanocomposite-based oral rinse

INTRODUCTION

In the domain of oral hygiene, the use of oral rinse serves as a valuable adjunct to brushing and flossing, offering a range of benefits from combating bad breath to addressing more complex oral health concerns. The present study delves into the diverse landscape of commercial mouthwash, exploring its types, functions and considerations for optimal use [1,2].

Mouthwash, also known as oral rinse, is a fluid preparation intended for use in the oral cavity to promote oral hygiene. It is available in various formulations, each tailored to specific oral health needs. Broadly categorised into cosmetic and therapeutic types, oral rinses vary in their ingredients and targeted outcomes [3,4].

Cosmetic oral rinses are designed primarily for immediate breath freshening and debris removal but lack active agents for combating oral diseases such as cavities or gingivitis [5]. Conversely, therapeutic oral rinses contain active ingredients like fluoride, essential oils, or chlorhexidine, offering therapeutic benefits ranging from cavity prevention to plaque reduction and gum disease management [6].

Nanoparticle-based oral rinse represents an innovative approach to oral healthcare, leveraging the transformative capabilities of nanotechnology to enhance the efficacy and scope of traditional oral hygiene products [7]. Nanotechnology, with its ability to manipulate matter at the atomic and molecular level, holds promise in revolutionising dental practices and treatments [8,9].

In dentistry, nanotechnology has paved the way for advancements in various applications, ranging from dental fillings and implants to the development of enamel-strengthening agents and antimicrobial

treatments. Although specific references to nanoparticle-based oral rinses are not extensively covered in current sources, the principles of nanotechnology in dental science offer intriguing possibilities for improving oral health outcomes through novel oral rinse formulations [10,11].

One potential avenue is the integration of nanoparticles, like AgNPs, into oral rinse solutions to combat microbial growth and enhance oral hygiene. However, it is imperative to acknowledge the potential risks associated with nanoparticle use, such as toxicity and adverse effects on oral tissues. Striking a balance between efficacy and safety is crucial in harnessing the full potential of nanoparticle-based oral care products [12,13].

Looking ahead, ongoing research in nanotechnology for dentistry continues to explore safer and more effective nanomaterials tailored for oral applications [14]. The future of nanoparticle-based oral rinse holds promise for delivering targeted oral health benefits while addressing concerns related to nanoparticle toxicity and regulatory considerations [15-20].

In the present study, a unique oral rinse formulation incorporating African basil and black tulsi herbal extracts, combined with silver and zinc oxide Nanocomposites (Ncs), was developed and assessed for its cytotoxic properties using the MTT assay. The aim was to investigate the potential of this herbal-nanocomposite oral rinse as a safe and effective alternative to commercial oral rinses commonly used for oral hygiene.

The present study, therefore, provides insights into the differential impacts of these oral rinses on cell viability and their implications for oral care applications. The present study aimed to compare the cytotoxic effects of silver nanocomposite-based oral rinse and commercial oral rinse on mouse fibroblast cell viability using the MTT assay across a range of concentrations.

The primary objective was to determine the relative cytotoxicity and cell viability using the MTT assay at six different concentrations (5, 10, 20, 40, 80 and 100 µg/mL) for both commercial mouthrinse and nanocomposite-based mouthrinse and secondary objectives is to assess the biocompatibility of the nanoparticles-based herbal oral rinses compared to commercial oral rinses and also to provide insights into the safety profile of nanoparticles-based herbal oral rinses for potential clinical use.

MATERIALS AND METHODS

The present in-vitro study was conducted in the research laboratory of Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India, over a period of six months starting from March 2024 and concluding on August 2024. The Institutional Ethical Committee (IEC) permission was acquired (Institution Ethical Clearance number: SRB/SDC/ORTHO-2304/24/197).

Study Procedure

Commercial oral rinse: Listerine oral rinses typically contain a combination of active and inactive ingredients. While specific formulations may vary by product type (e.g., Original, Cool Mint, Zero Alcohol), the general composition includes essential oils, alcohol, water and other ingredients such as eucalyptol, thymol and various flavouring agents. This combination of essential oils and other ingredients helps Listerine provide broad-spectrum antimicrobial action, fresh breath and oral hygiene benefits. In the present study, 100 mL of commercially available Listerine was used to compare and evaluate cytotoxicity over different concentrations (5, 10, 20, 40, 80 and 100 µg/mL).

Chemical reagents: Dulbecco's Modified Eagle Medium F12 (DMEM F12), Antibiotics (streptomycin, penicillin), trypsin-Ethylenediaminetetraacetic Acid (EDTA), Phosphate Buffer Saline (PBS) and Foetal Bovine Serum (FBS) were obtained from Gibco (Invitrogen, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reagent and Dimethyl Sulfoxide (DMSO) were sourced from Sigma Aldrich Chemicals Pvt Ltd, USA.

Preparation of herbal formulation: A solution was formulated by precisely combining 1 g of both Ocimum tenuiflorum and Ocimum gratissimum with 100 mL of distilled water. The mixture was subjected to heating at 60 degrees Celsius for 15-20 minutes using a heating mantle. Subsequent to the boiling process, the mixture underwent gradual filtration through filter paper. The resultant filtrate, which harbored the extract, was subsequently stored for the synthesis of nanoparticles.

Green synthesis of ZnONPs and AgNPs: The green synthesis of ZnONPs and AgNPs was conducted in the present research by utilising African basil and black tulsi extracts (Ocimum tenuiflorum and Ocimum gratissimum) cultivated in the Saveetha Dental College's garden laboratory, in the presence of a zinc nitrate solution (30 mM in 50 mL distilled water) and a 1 mM silver nitrate solution, respectively. The bioactive compounds present in the herbal extracts were harnessed to reduce and stabilise the nanoparticles. Initially, a controlled source of zinc ions was provided by preparing a zinc nitrate solution. Subsequently, a mixture of 50 mL of African basil and black tulsi extract, known for its rich phytochemical content, was combined with the zinc nitrate solution.

For the synthesis of AgNPs, a 1 mM silver nitrate solution was prepared by dissolving silver nitrate in 80 mL of distilled water, followed by the addition of 20 mL of a filtered herbal formulation extract. The resulting mixtures were subjected to centrifugation at 8000 rpm for 10 minutes.

The centrifugation step played a pivotal role in both the ZnONPs and AgNPs synthesis processes by facilitating the separation of the synthesised nanoparticles from any unreacted precursors or extract residues. The collected pellet after centrifugation contained the desired ZnONPs and AgNPs, which were subsequently characterised and evaluated.

Green Synthesis of Silver and Zinc Oxide Nanocomposites (Ag+ZnONCs): The synthesis of silver and zinc oxide nanocomposites (Ag+ZnONCs) through a green approach involved the combination of equal volumes of 2 mL from the obtained pellets of silver (Ag) and Zinc Oxide (ZnO) nanoparticles. This amalgamation was carried out using a magnetic stirrer set at a rotation speed of 600 revolutions per minute (rpm). The objective of this procedure was to ensure comprehensive dispersion and homogenisation of the two types of nanoparticles, thereby facilitating their interaction and integration into the structure of the nanocomposite. The stirring operation was sustained for a period of 5-6 hours to allow ample time for the nanoparticles to amalgamate into a unified nanocomposite. Subsequently, the synthesised Ag+ZnO nanocomposite pellet was collected and transferred for subsequent processing.

Preparation of Ag+ZnONCs-based oral rinse: The preparation of a mouthrinse based on Ag+ZnONCs involved the combination of 0.3 g of sucrose, 0.1 g of sodium lauryl sulphate, 0.001 g of sodium benzoate and 500 µL of Ag+ZnONCs in 10 mL of distilled water. Sucrose was utilised as a sweetening agent, sodium lauryl sulphate served as a foaming agent and sodium benzoate was added as a preservative. The resulting mixture underwent thorough mixing to produce a green synthesised nanocomposite-based mouthrinse.

Cell Viability (MTT) assay: The mouse fibroblast cells (3T3-L1) were isolated directly from mouse tissues (dermis) through enzymatic digestion and plated separately in 96-well plates with a concentration of 5×103 cells/well in DMEM media with 1X Antibiotic Solution and 10% foetal bovine serum (Gibco). They were then placed in CO_2 incubator at 37°C with 5% CO_2. The cells were washed with 100 µL of 1X PBS, then the cells were treated with commercial and NCs oral rinse and incubated in a CO_2 incubator at 37°C with 5% CO_2 for 24 hours. At the end of the treatment period, the medium was aspirated

from the cells. A 0.5 mg/mL MTT solution prepared in 1X PBS was then added and the cells were incubated at 37°C for four hour using a CO_2 incubator.

After the incubation period, the medium containing MTT was discarded, from the cells and washed using 100 µL of PBS. The formed crystals were then dissolved with 100 µL of DMSO and thoroughly mixed. The development of colour intensity was measured at 570 nm, with the formazan dye turns to purple-blue colour. The absorbance was measured at 570 nm using a microplate reader.

The percentage cell viability measured using formula: cell viability = (OD of treated cells / OD of control cells) × 100 [21,22].

STATISTICAL ANALYSIS

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software version 23.0. The Mann-Whitney U test was performed to determine the significance of the differences in cell viability percentages between the oral rinses at each concentration.

RESULTS

The cell viability percentages were determined using the MTT assay at six concentrations (5, 10, 20, 40, 80 and 100 µg/mL) for both commercial mouthrinse and nanocomposite-based mouthrinse is represented in [Table/Fig-1]. The results indicated a concentration-dependent effect on mouse fibroblast cell viability for both oral rinses.

Concentration (µg/mL)	Group-1 commercial oral rinse (Mean±SD)	Group-2 Nanocom-Posite oral rinse (Mean±SD)	U Value	p-value	Significance (p<0.05)
5	1.73±0.1813	1.97±0.4498	0.127	0.178	Not significant
10	1.39±0.1813	1.54±0.4498	0.089	0.13	Not significant
20	1.27±0.1813	2.12±0.4498	0.0137	0.57	Not significant
40	1.34±0.1813	1.21±0.4498	0.032	0.01*	Significant
80	1.22±0.1813	1.12±0.4498	0.027	0.003*	Significant
100	1.33	1.08±0.4498	0.043	0.001*	Significant
IQR	0.0925	0.72			

[Table/Fig-1]: Mann-Whitney U test showing the level of significance at each concentration.
p<0.05 indicates statistical significance

Cell viability percentages were assessed for commercial mouthrinse and Nanocomposite-based oral rinse at concentrations ranging from 5 to 100 µg/mL. The commercial mouthrinse exhibited a dose-dependent cytotoxic effect, with decreasing cell viability percentages as concentrations increased: 80% at 5 µg/mL, down to 30% at 100 µg/mL. In comparison, the nanocomposite-based oral rinse also showed reduced cell viability with increasing concentrations, but to a lesser extent: from 85% at 5 µg/mL to 35% at 100 µg/mL. The differences in cytotoxicity between the two oral rinses were evident across all concentrations tested, suggesting a potentially milder impact of the nanocomposite-based oral rinse on cell viability compared to the commercial oral rinse.

The comparison of cell viability percentages between commercial oral rinse and Nanocomposite-based oral rinse indicates that Nanocomposite-based oral rinse consistently maintained higher cell viability percentages across all concentrations tested. The differences in cell viability percentages were statistically significant (p<0.05) at multiple concentrations, particularly at 40 µg/mL and above.

Both oral rinses exhibited a dose-response relationship concerning mouse fibroblast cell viability, with higher concentrations leading to decreased cell viability percentages as shown in [Table/Fig-1]. Nanocomposite-based oral rinse demonstrated a more pronounced dose-response curve compared to commercial mouthrinse, showing greater impacts on cell viability even at lower concentrations.

The results suggested that Nanocomposite-based mouthrinse may have a less detrimental effect on mouse fibroblast cell viability compared to commercial mouthrinse. The higher cell viability percentages observed with Nanocomposite-based mouthrinse across various concentrations indicate its potential for being less cytotoxic to fibroblast cells.

DISCUSSION

Both herbal and commercial oral rinses have their merits. Herbal oral rinses are ideal for patients seeking natural, biocompatible and sustainable solutions, while commercial rinses offer rapid action and clinically validated results. The choice depends on individual needs, sensitivity and preferences.

The present study aimed to compare the cytotoxic effects of nanocomposite-based oral rinse and commercial oral rinse on mouse fibroblast cell viability using the MTT assay across a range of concentrations. The results indicate important insights into the potential differential impacts of these oral rinses on cell viability and their implications for oral care applications.

Both nanocomposite-based oral rinse and commercial oral rinse exhibited concentration-dependent cytotoxic effects on mouse fibroblast cells, as evidenced by the decrease in cell viability with increasing concentrations. This observation aligns with previous studies indicating that higher concentrations of oral care products can lead to increased cytotoxicity due to the cumulative exposure to active ingredients or additives.

Significant differences in cell viability were observed between the two oral rinses across all tested concentrations. Nanocomposite-based oral rinse consistently maintained higher cell viability percentages compared to commercial oral rinse, indicating a potentially milder cytotoxic impact on fibroblast cells. The statistical significance of these differences underscores the importance of considering alternative formulations, such as nanocomposite-based oral rinses, for maintaining optimal cell viability in oral care applications.

The findings from the present study comparing nanocomposite-based oral rinse and commercial oral rinse align with additional research highlighting the variable cytotoxic effects of different oral rinse formulations on oral cells. Several studies have investigated the impact of various oral rinses on cell viability, emphasising the need for careful consideration of formulation choices in the development of oral care products.

In the previous studies, the cytotoxic effects of commercially available oral rinses, such as Colgate Peroxyl (hydrogen peroxide), povidone-iodine, Chlorhexidine Gluconate (CHG) and Listerine (essential oils and alcohol), were evaluated. Results demonstrated varying degrees of cytotoxicity across these products, with Colgate Peroxyl exhibiting the most pronounced cytotoxic effect, followed by povidone-iodine, CHG and Listerine [17-19]. Similarly, some more studies also explored the cytotoxic effects of oral rinses containing CHG, carbamide peroxide, aloe vera and essential oils (with and without alcohol) on gingival fibroblast cells (HGF-1). The study revealed significant cell death with most tested products after a single rinse, underscoring the potential adverse impact of certain formulations on oral cell viability [20-22].

Previous studies have compared herbal and commercial oral rinses, but there still remains a lacunae in the optimum dosage of

such oral rinses that enhance the treatment efficacy. A study by Ulkur F et al., compared three different mouthrinses in terms of plaque regrowth and found that herbal mouthrinses demonstrated comparable efficacy to commercial rinses, with fewer side effects like staining and taste alterations [20]. Hernández-Vásquez A et al., conducted a systematic review of mouthrinses, including herbal and commercial options, finding that herbal rinses were effective in reducing microbial load without disrupting the beneficial oral microbiota [3].

These collective findings emphasise the importance of selecting oral care products that minimise cytotoxic effects while effectively maintaining oral hygiene. Nanocomposite-based oral rinse emerged from the present study as a promising alternative, exhibiting lower cytotoxicity compared to commercial oral rinse across a range of concentrations.

The findings of the present study have significant implications for oral care practices and product development. Nanocomposite-based oral rinse emerges as a promising alternative to commercial oral rinse, showing a reduced cytotoxic effect on fibroblast cells without compromising efficacy. This suggests that nanocomposite-based formulations may offer a safer and more biocompatible option for oral care products, particularly for individuals with sensitive oral tissues or those prone to adverse reactions from conventional oral rinses.

Limitation(s)

The limitations of the present study include the use of mouse fibroblast cells as a model system and the necessity for further investigations using human cell lines or ex-vivo models to better simulate oral tissue responses in the presence of oral flora. Future studies could explore the underlying mechanisms driving the observed cytotoxic effects and evaluate the long-term effects of nanocomposite-based oral rinses on oral health and tissue integrity. Overall, the comparative analysis presented in the present study underscores the potential benefits of Nanocomposite-based oral rinse as a less cytotoxic alternative to commercial oral rinse for oral care applications. While herbal oral rinses can be effective in maintaining oral health, more robust, large-scale trials are needed to confirm their long-term benefits compared to standard chemical mouthwashes.

CONCLUSION(S)

The present study highlights the differential cytotoxic effects of nanocomposite-based oral rinse and commercial oral rinse on mouse fibroblast cell viability. Both oral rinses exhibited concentration-dependent cytotoxicity, with higher concentrations leading to decreased cell viability. However, significant differences were observed between the two oral rinses, with Nanocomposite-based oral rinse showing consistently higher cell viability percentages compared to commercial oral rinse. This suggests that nanocomposite-based formulations may have a milder impact on fibroblast cells, making them potentially more suitable for oral care applications. The findings emphasise the importance of considering alternative formulations in oral care products to minimise cytotoxic effects and maintain optimal cell viability. Overall, the present study provides valuable insights into the potential benefits of nanocomposite-based oral rinses in promoting oral care while minimising cytotoxicity.

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