Original Article

APIGENIN-FUNCTIONALIZED ZINC OXIDE NANOPARTICLES FOR ORAL DISEASE MANAGEMENT: AN IN VITRO ASSESSMENT

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ABSTRACT

Nanotechnology has advanced human health and medicine through the development of multifunctional biomaterials. Zinc oxide nanoparticles (ZnO NPs) possess antimicrobial, anti-inflammatory, and antioxidant properties, making them promising candidates for oral disease prevention and management. Functionalization with apigenin may further enhance their therapeutic potential for disease management and wellbeing. To prepare apigenin-functionalized ZnO NPs and evaluate their cytotoxicity, antimicrobial activity against oral pathogens, anti-inflammatory, and antioxidant properties. Apigenin-functionalized ZnO NPs were prepared by dispersing zinc oxide powder in apigenin solution. Morphology was analyzed by SEM. Cytotoxicity was assessed using the brine shrimp lethality assay. Antimicrobial activity was tested against *Staphylococcus aureus*, *Streptococcus mutans*, and *Enterococcus faecalis*. Anti-inflammatory and antioxidant activities were evaluated using BSA denaturation and DPPH assays, respectively. SEM showed quasi-spherical nanoparticles with mild aggregation. Cytotoxicity testing indicated favorable biocompatibility. Antimicrobial activity increased with concentration, with maximum inhibition against *S. mutans* (12 mm at 100 µL). Anti-inflammatory activity reached 75%, while antioxidant scavenging activity reached 82% at 100 µL. Apigenin-functionalized ZnO nanoparticles demonstrated promising biocompatibility and biological activity, suggesting potential applications in oral disease management, medicine, and infectious disease control. Further in vivo studies are required.

Key words: Metal oxide, Nanoparticle, Nanotechnology, Infectious disease, Wellbeing.

Introduction

Nanotechnology has emerged as a transformative scientific discipline owing to its ability to engineer materials at the nanoscale with unique physicochemical and biological properties [1-4]. Among the diverse nanomaterials investigated for biomedical applications, zinc oxide nanoparticles (ZnO NPs) have attracted remarkable attention because of their excellent antimicrobial potential, biocompatibility, photocatalytic activity, anti-inflammatory effects, and cost-effective synthesis [5, 6]. Their high surface area-to-volume ratio and reactive surface characteristics make them suitable candidates for therapeutic and diagnostic purposes.

Surface functionalization of nanoparticles using naturally derived bioactive compounds has emerged as an effective strategy to enhance biological performance while reducing dependence on synthetic additives [7]. Phytochemicals can improve nanoparticle stability and may impart additional antioxidant, antimicrobial, and anti-inflammatory properties [8].

Apigenin is a naturally occurring flavonoid abundantly present in parsley, chamomile, celery, onions, and several medicinal plants. It has been widely studied for its potent pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, anticancer, and wound healing properties [9]. Apigenin exerts anti-inflammatory effects through modulation of nuclear factor-kappa B (NF-κB), cyclooxygenase-2 (COX-2), and pro-inflammatory cytokines. Additionally, its free radical scavenging capacity and ability to regulate oxidative stress pathways make it an attractive bioactive compound for therapeutic applications [10].

Oral diseases such as dental caries, periodontal disease, peri-implant infections, and endodontic infections remain major global public health concerns affecting human health and wellbeing [11-15]. Microorganisms, including *Streptococcus mutans* and *Enterococcus faecalis*, are frequently associated with cariogenic biofilms and persistent endodontic infections [16]. Therefore, multifunctional nanomaterials with antimicrobial and anti-inflammatory properties may be valuable for the prevention and management of oral diseases [17-20].

Although ZnO NPs and apigenin have individually demonstrated promising biological properties, limited evidence is available regarding apigenin-functionalized ZnO NPs and their combined bioactivity. Therefore, the present in vitro study aimed to prepare and characterize apigenin-functionalized ZnO NPs and evaluate their cytotoxicity, antimicrobial efficacy, anti-inflammatory

activity, and antioxidant potential.

Materials and Methods

Materials

Apigenin (≥98% purity) was procured from Sigma-Aldrich. Zinc oxide powder, bovine serum albumin (BSA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol, hydrochloric acid, sodium chloride, and other analytical grade reagents were obtained from standard commercial sources. All solutions were prepared using distilled water.

Preparation of apigenin solution

A stock solution of apigenin was prepared by dissolving 10 mg of commercially purchased apigenin in 100 mL of distilled water under continuous magnetic stirring. Mild heating at 50°C was applied to facilitate dissolution. The prepared solution was filtered through Whatman No. 1 filter paper and stored in an amber-colored container under refrigerated conditions until further use.

Synthesis of apigenin-functionalized NPs

A quantity of 0.1 g zinc oxide powder was dispersed in 100 mL of the prepared apigenin solution and subjected to continuous magnetic stirring at room temperature. The dispersion was incubated for 24–36 h under dark conditions to minimize photo-degradation of apigenin and to facilitate surface interaction between apigenin and ZnO particles. The resulting suspension was centrifuged at 7000 rpm for 10 min. The pellet was washed with distilled water to remove unbound residues and centrifuged again. The purified apigenin-functionalized ZnO NPs were redispersed in distilled water and stored in sterile airtight Eppendorf tubes for subsequent analyses.

Characterization of nanoparticles

Scanning Electron Microscopy (SEM)

Morphological characteristics of the prepared nanoparticles were evaluated using scanning electron microscopy. Samples were dried, mounted on specimen stubs, and sputter-coated with a thin gold layer to improve conductivity. The coated samples were analyzed using a field-emission scanning electron microscope (JEOL JSM-IT 800; JEOL, Peabody, MA, USA) to assess particle shape, surface morphology, and aggregation pattern.

Cytotoxicity assay

Cytotoxicity was assessed by the brine shrimp lethality assay. Artificial seawater was prepared using sea salt in distilled water. Brine shrimp eggs were incubated for 48 hr under aeration and illumination to obtain nauplii. Ten nauplii were transferred into each well containing saline solution. Different concentrations of apigenin-functionalized ZnO NPs (25 µL, 50 µL, and 100 µL) were added. A control group containing only nauplii in saline solution was maintained. After 24 hr, surviving nauplii were counted, and percentage mortality was calculated.

Antimicrobial activity

Antibacterial efficacy was evaluated using the agar well diffusion technique against *Staphylococcus aureus*, *Streptococcus mutans*, and *Enterococcus faecalis*. The selected microbial strains were chosen based on their relevance to oral infectious diseases. Sterile Mueller-Hinton agar plates were inoculated with respective microbial cultures. Wells were prepared and filled with 25 µL, 50 µL, and 100 µL of synthesized nanoparticle suspension. Standard antibiotic discs served as positive controls. Plates were incubated at 37°C for 24 hr, following which zones of inhibition were measured in millimeters.

Anti-inflammatory activity

Anti-inflammatory activity was determined using the BSA denaturation assay. Various concentrations of apigenin-functionalized ZnO NPs (25 µL, 50 µL, and 100 µL) were mixed with 1% BSA solution. The pH was adjusted to 6.8 using 1N hydrochloric acid. Samples were incubated at room temperature for 20 min, then heated. After cooling, absorbance was recorded at 660 nm. Diclofenac sodium served as the standard control.

The percentage inhibition was calculated using:

$$\% \text{ Inhibition} = [(\text{Absorbance of control} - \text{Absorbance of sampl} / \text{Absorbance of control}] \times 100 \quad (1)$$

Antioxidant activity

Antioxidant potential was assessed using the DPPH free radical scavenging assay. Different concentrations of nanoparticles were mixed with 1 mL of 0.1 mM DPPH solution and Tris-HCl buffer (pH 7.4). The reaction mixture was incubated in dark conditions for 30 min. Absorbance was measured at 517 nm. Butylated hydroxytoluene served as the standard control.

The scavenging activity was calculated using:

$$\% \text{ Scavenging} = [(\text{Absorbance of control} - \text{Absorbance of sampl} / \text{Absorbance of control}] \times 100 \quad (2)$$

Results and Discussion

Topography analysis

The SEM micrograph of apigenin-functionalized ZnO NPs demonstrated the formation of predominantly quasi-spherical to slightly irregular nanoparticles with a tendency to form agglomerated clusters, likely due to the high surface energy of ZnO and intermolecular interactions facilitated by apigenin. The particles appeared within the nanoscale range and show relatively uniform distribution despite localized aggregation. Notably, the surface morphology was rough and heterogeneous, indicating successful functionalization and capping of ZnO NPs by apigenin, which may enhance

surface reactivity and biological interactions. Overall, the observed morphology confirmed the successful synthesis of apigenin-functionalized ZnO NPs with structural characteristics suitable for improved bioactivity and potential biomedical applications **(Figure 1)**.

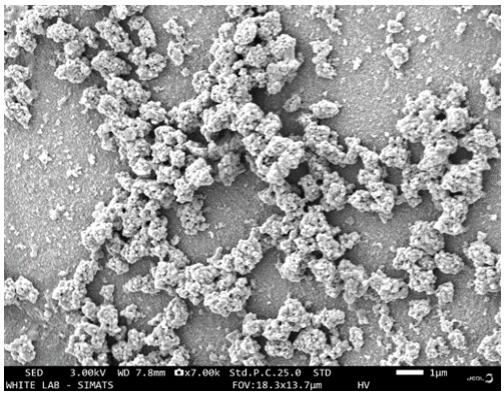

Figure 1. SEM of apigenin-functionalized ZnO NPs

Cytotoxic assay

On day 1, all groups showed 10 live nauplii at 25 µL, 50 µL, and 100 µL, indicating no immediate toxic effect. On day 2, 10 live nauplii were observed at 25 µL, while the number decreased slightly to 9 at 50 µL and 8 at 100 µL. The findings indicate a mild concentration-dependent reduction in viability over time, while overall survival remained high, suggesting favorable biocompatibility of the synthesized nanoparticles **(Figure 2)**.

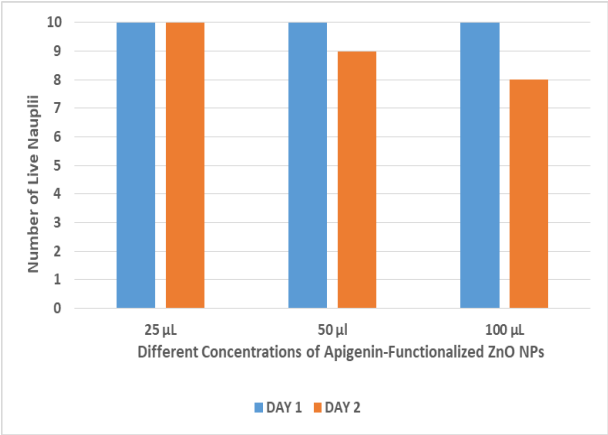

Figure 2. Cytotoxicity assessment of apigenin-functionalized ZnO NPs using brine shrimp lethality assay

Antimicrobial activity

The antimicrobial activity of apigenin-functionalized ZnO NPs increased in a concentration-dependent manner against all tested microorganisms. Against *S. aureus*, the zone of inhibition increased from 6 mm at 25 µL to 8 mm at 50 µL and 10 mm at 100 µL. For *S. mutans*, inhibition zones of 6 mm, 8 mm, and 12 mm were observed at 25 µL, 50 µL, and 100 µL, respectively. Against *E. faecalis*, the corresponding zones measured 5 mm, 8 mm, and 10 mm. The antibiotic control showed inhibition zones of 24 mm against *S. aureus*, 26 mm against *S. mutans*, and 22 mm against *E. faecalis*. Overall, the findings indicate progressive antibacterial efficacy with increasing nanoparticle concentration **(Table 1)**.

Table 1. Antimicrobial activity of apigenin-functionalized ZnO NPs

Concentration (µL)	Zone of inhibition (mm)		
	S. aureus	***S. mutans***	***E. faecalis***
25	6	6	5
50	8	8	8
100	10	12	10
Antibiotic	24	26	22

Anti-inflammatory activity

The anti-inflammatory assay demonstrated a concentration-dependent increase in percentage inhibition by apigenin-functionalized ZnO NPs. At 25 µL, the percentage inhibition was 45% for the nanoparticle group compared with 50% for the standard control. At 50 µL, inhibition increased to 60% for the nanoparticle group and 65% for the control. The highest anti-inflammatory activity was observed at 100 µL, with 75% inhibition for apigenin-functionalized ZnO nanoparticles and 80% for the control. Overall, the synthesized nanoparticles exhibited substantial anti-inflammatory activity comparable to the standard, with enhanced efficacy at higher concentrations **(Figure 3)**.

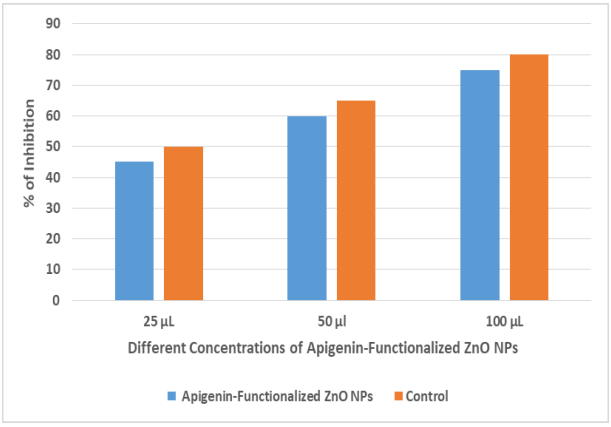

Figure 3. Comparison of anti-inflammatory activity of apigenin-functionalized ZnO NPs and standard control at different concentrations

Antioxidant activity

The antioxidant assay demonstrated a concentration-dependent increase in the percentage of scavenging activity of apigenin-functionalized ZnO NPs. At 25 µL, the percentage of scavenging was 64% for the nanoparticle group compared with 68% for the standard control. At 50 µL, scavenging activity increased to 74% for the nanoparticle group and 78% for the control. The highest scavenging percentage was observed at 100 µL, with 82% for apigenin-functionalized ZnO NPs and 86% for the

control. Overall, the synthesized nanoparticles exhibited strong free radical scavenging potential comparable to the standard, with enhanced activity at higher concentrations **(Figure 4)**.

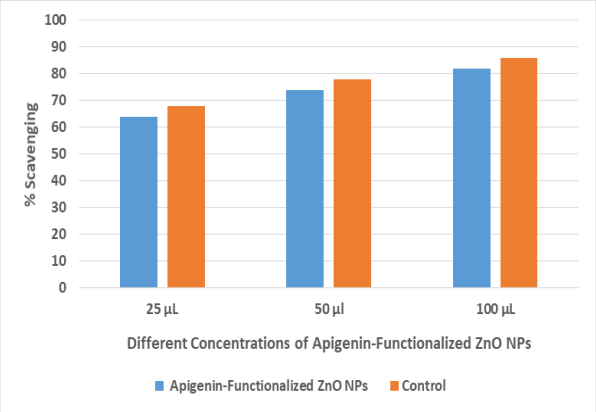

Figure 4. Comparison of the percentage of scavenging activity between apigenin-functionalized ZnO NPs and standard control at different concentrations

The present study evaluated the biological properties of apigenin-functionalized ZnO NPs. The findings demonstrated successful nanoparticle formation with favorable morphology, minimal cytotoxicity, and appreciable antimicrobial, anti-inflammatory, and antioxidant activities. These observations indicate that apigenin-functionalized ZnO NPs may serve as a promising bioactive nanomaterial for future biomedical applications.

Scanning electron microscopic analysis confirmed the successful synthesis of nanoparticles with well-defined morphology and uniform structural characteristics. The particles appeared relatively discrete with mild aggregation, which is commonly observed in biosynthesized metal oxide nanoparticles due to interparticle surface interactions. The SEM findings suggest that apigenin may have functioned as both a reducing and stabilizing agent during nanoparticle formation [21], thereby assisting in controlled structural development.

Biocompatibility is an essential consideration for any nanoparticle intended for therapeutic use. In the present study, the brine shrimp lethality assay demonstrated excellent biocompatibility, with negligible toxicity observed across concentrations. At 25 µL, all 10 nauplii remained viable on the second day, whereas 9 and 8 nauplii survived at 50 µL and 100 µL, respectively. These findings indicate only a mild concentration-dependent reduction in survival, suggesting acceptable biosafety of the synthesized nanoparticles within the tested range. The favorable cytocompatibility may be attributed to the natural bioactive nature of apigenin and the controlled synthesis process.

The synthesized nanoparticles exhibited notable antibacterial activity against all tested oral pathogens. Against *Staphylococcus aureus*, the zones of inhibition measured 6 mm, 8 mm, and 10 mm at 25 µL, 50 µL, and 100 µL, respectively. For *Streptococcus mutans*, inhibition zones of 6 mm, 8 mm, and 12 mm were observed, while *Enterococcus faecalis* demonstrated zones of 5 mm, 8 mm, and 10 mm at corresponding concentrations. Although the standard antibiotic showed larger inhibition zones of 24 mm, 26 mm, and 22 mm, the nanoparticle formulation demonstrated a clear dose-dependent antibacterial response. This antimicrobial activity may be related to membrane disruption, generation of reactive oxygen species, release of zinc ions, and bioactive contribution from apigenin [22].

Kim S *et al*. reported that apigenin induced apoptosis-like bacterial death in *Escherichia coli* through elevation of intracellular calcium, increased reactive oxygen and reactive nitrogen species, membrane lipid damage, glutathione oxidation, and DNA injury [23]. These findings support the possibility that apigenin coating enhances the antibacterial potential of ZnO nanoparticles through multiple synergistic mechanisms. Therefore, the antibacterial effects observed in the present study may result from the combined action of zinc oxide nano-structures and flavonoid-functionalized oxidative stress on microbial cells.

The present study also demonstrated marked anti-inflammatory activity, with responses comparable to the standard control. This finding is in accordance with the established pharmacological effects of apigenin. Patil RH *et al*. demonstrated that apigenin significantly inhibited lipopolysaccharide-induced inflammatory mediators, including inducible nitric oxide synthase, cyclooxygenase-2, nitric oxide production, and cytokines such as IL-1β, IL-2, IL-6, IL-8, and TNF-α. They also reported suppression of AP-1 signaling proteins, confirming the anti-inflammatory mechanism of apigenin at the molecular level [24]. The current results suggest that apigenin retained its anti-inflammatory activity following nanoparticle synthesis.

Antioxidant analysis in the present study revealed strong free radical scavenging activity, which was comparable to the standard antioxidant control. This may be attributed to the hydrogen-donating and radical-neutralizing properties of apigenin, along with the high reactive surface area of ZnO nanoparticles. Cicek M *et al*. demonstrated that apigenin significantly reduced lipid peroxidation and restored antioxidant defense systems such as superoxide dismutase, catalase, and glutathione in a rat sepsis model [25]. These observations support the antioxidant effects identified in the present investigation.

Further evidence was provided by Karamese M *et al*., who reported that apigenin reduced pro-inflammatory cytokines, suppressed NF-κB activation, increased IL-10 levels, and normalized oxidative stress parameters in spleen tissue during experimental sepsis [26]. Such findings reinforce the multifunctional therapeutic value of apigenin and justify its incorporation into nanoparticle systems.

Collectively, the present study suggests that apigenin-functionalized ZnO NPs possess combined antimicrobial, anti-inflammatory, antioxidant, and biocompatible properties. The synergistic interaction between the ZnO nanoparticle core and apigenin surface constituents may be beneficial for biomedical applications such as periodontal therapy, implant coatings, oral wound healing, and local drug delivery.

The demonstrated activity against *Streptococcus mutans* and *Enterococcus faecalis*, together with anti-inflammatory and antioxidant effects, suggests that apigenin-functionalized ZnO NPs may have potential applications in oral disease conditions characterized by microbial challenge and tissue inflammation, including dental caries, endodontic infections, periodontal disease, and peri-implant disease.

However, the present investigation was limited to in vitro evaluation. Further studies involving mammalian cell lines, mechanistic molecular assays, release kinetics, and in vivo validation are necessary before clinical translation of apigenin-functionalized ZnO NPs can be considered [27-30].

Conclusion

Within the limitations of this in vitro study, apigenin-functionalized ZnO NPs were successfully synthesized and exhibited favorable morphological characteristics on SEM analysis. The nanoparticles demonstrated minimal cytotoxicity, concentration-dependent antimicrobial activity, and appreciable anti-inflammatory and antioxidant properties. These findings suggest that apigenin-functionalized ZnO NPs may hold potential for future oral disease management and broader biomedical applications.

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Conflict of interest: None

Financial support: None

Ethics statement: The study protocol was approved by the Institutional Ethics Committee, Saveetha Dental College and Hospitals, Chennai, India.

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