EMBRYONIC TOXICOLOGY EVALUATION OF LEMON GRASS AND GINGER FORMULATION MEDIATED NANO ZINCOXIDE PARTICLES - CHITOSEN NANO COMPOSTE WASTE DENTAL VARNISH.

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Introduction

The emergence of antimicrobial resistance and the increasing demand for safe, biocompatible, and sustainable dental materials have encouraged researchers to explore plant-mediated nanotechnology for oral healthcare applications. Conventional antimicrobial agents used in dentistry are often associated with undesirable side effects, including microbial resistance, cytotoxicity, and limited long-term effectiveness. Green synthesis of nanoparticles has therefore gained significant attention as an eco-friendly and cost-effective alternative to conventional chemical and physical synthesis methods. This approach utilizes naturally occurring phytochemicals present in plant extracts as reducing and stabilizing agents, eliminating the need for hazardous chemicals while producing nanoparticles with enhanced biological properties. Compared with chemically synthesized nanoparticles, green-synthesized nanoparticles demonstrate improved biocompatibility, lower toxicity, greater stability, and enhanced antimicrobial activity, making them suitable for various biomedical and dental applications (1,2).

Among the various metal oxide nanoparticles, zinc oxide nanoparticles (ZnO NPs) have emerged as one of the most promising nanomaterials because of their broad-spectrum antimicrobial, antioxidant, anti-inflammatory, and wound-healing properties. Zinc is an essential trace element involved in numerous physiological processes and is considered relatively safe for biomedical use. ZnO nanoparticles exhibit excellent antibacterial activity against both Gram-positive and Gram-negative microorganisms through the generation of reactive oxygen species, disruption of bacterial cell membranes, and release of zinc ions that interfere with microbial metabolism. These properties have led to their incorporation into restorative materials, dental varnishes, root canal sealers, implant coatings, adhesives, and drug delivery systems to reduce bacterial colonization, inhibit biofilm formation, and improve the longevity of dental restorations (3,4).

The biological properties of ZnO nanoparticles can be further enhanced through the incorporation of medicinal plant extracts. Lemongrass (*Cymbopogon citratus*) is a medicinal herb rich in citral, flavonoids, tannins, and phenolic compounds that exhibit potent antibacterial, antioxidant, and anti-inflammatory activities. Several studies have demonstrated its effectiveness against oral pathogens responsible for dental plaque, gingivitis, periodontal disease, and dental caries. Similarly, dry ginger (*Zingiber officinale*) contains biologically active compounds such as gingerols, shogaols, paradols, and zingerone, which possess significant antimicrobial, antioxidant, analgesic, and anti-inflammatory properties. These phytochemicals contribute to the inhibition of oral biofilm formation, reduction of plaque accumulation, suppression of inflammatory responses, and maintenance of periodontal health. The synergistic combination of lemongrass and ginger extracts with ZnO nanoparticles is expected to improve antimicrobial efficacy while minimizing toxicity, making the formulation a promising candidate for preventive dental care (5,6).

Chitosan, a naturally occurring deacetylated derivative of chitin, has attracted considerable interest in dentistry because of its biodegradability, biocompatibility, bioadhesive nature, and intrinsic antimicrobial activity. Due to its positive charge, chitosan readily interacts with negatively charged bacterial cell membranes, thereby inhibiting microbial growth. Furthermore, chitosan exhibits excellent film-forming ability, allowing prolonged adhesion to tooth surfaces and controlled release of therapeutic agents. These characteristics make chitosan an ideal carrier polymer for dental varnishes, enhancing the retention, stability, and sustained antimicrobial action of incorporated nanoparticles and plant-derived bioactive compounds (7).

Before any newly developed biomaterial can be considered for clinical use, its biological safety and developmental toxicity must be thoroughly evaluated. The zebrafish (*Danio rerio*) embryo model has become one of the most widely accepted vertebrate models for toxicity screening because of its rapid embryonic development, transparent embryos, high reproductive rate, ease of maintenance, and significant genetic homology with humans. Zebrafish embryos are highly sensitive to environmental pollutants and nanomaterials, enabling early detection of developmental toxicity. Assessment of parameters such as embryo survival, hatching rate, larval viability, and morphological abnormalities provides valuable information regarding the biocompatibility and safety of newly synthesized nanomaterials intended for biomedical applications (8,9).

Therefore, the present study aimed to synthesize green zinc oxide nanoparticles using dry ginger and lemongrass extracts and incorporate them into a chitosan-based dental varnish. The developmental toxicity and biocompatibility of the formulated varnish were evaluated using the zebrafish embryo model by assessing embryo mortality, hatching rate, larval viability, and morphological changes at different concentrations. The findings of this study may contribute to the development of a safe, eco-friendly, plant-based dental varnish with potential applications in the prevention and management of oral diseases.

Materials and methods

Dry ginger (*Zingiber officinale*) powder and lemongrass (*Cymbopogon citratus*) were boiled in distilled water to obtain the plant extract. The extract was mixed with 50 mL of 20 mM zinc nitrate hexahydrate solution for the green synthesis of zinc oxide nanoparticles. The reaction mixture was continuously stirred using a magnetic stirrer and centrifuged to obtain the synthesized nanoparticles. These nanoparticles were incorporated into a chitosan polymer matrix to formulate the dental varnish.

Healthy fertilized zebrafish (*Danio rerio*) embryos were collected and rinsed with E3 embryo medium. Viable embryos were selected and divided into control and experimental groups. The experimental groups were exposed to different concentrations of the dental varnish (1, 2, 4, 8, and 16 µL) from 24 to 96 hours post-fertilization (hpf), while the control group was maintained in E3 embryo medium.

Embryonic development was observed every 24 hours under a stereomicroscope. The parameters evaluated included embryo mortality, hatching rate, larval viability, and the presence of morphological abnormalities. Images of the developing embryos were captured using a stereomicroscope for documentation and comparison among the different experimental groups.

Results

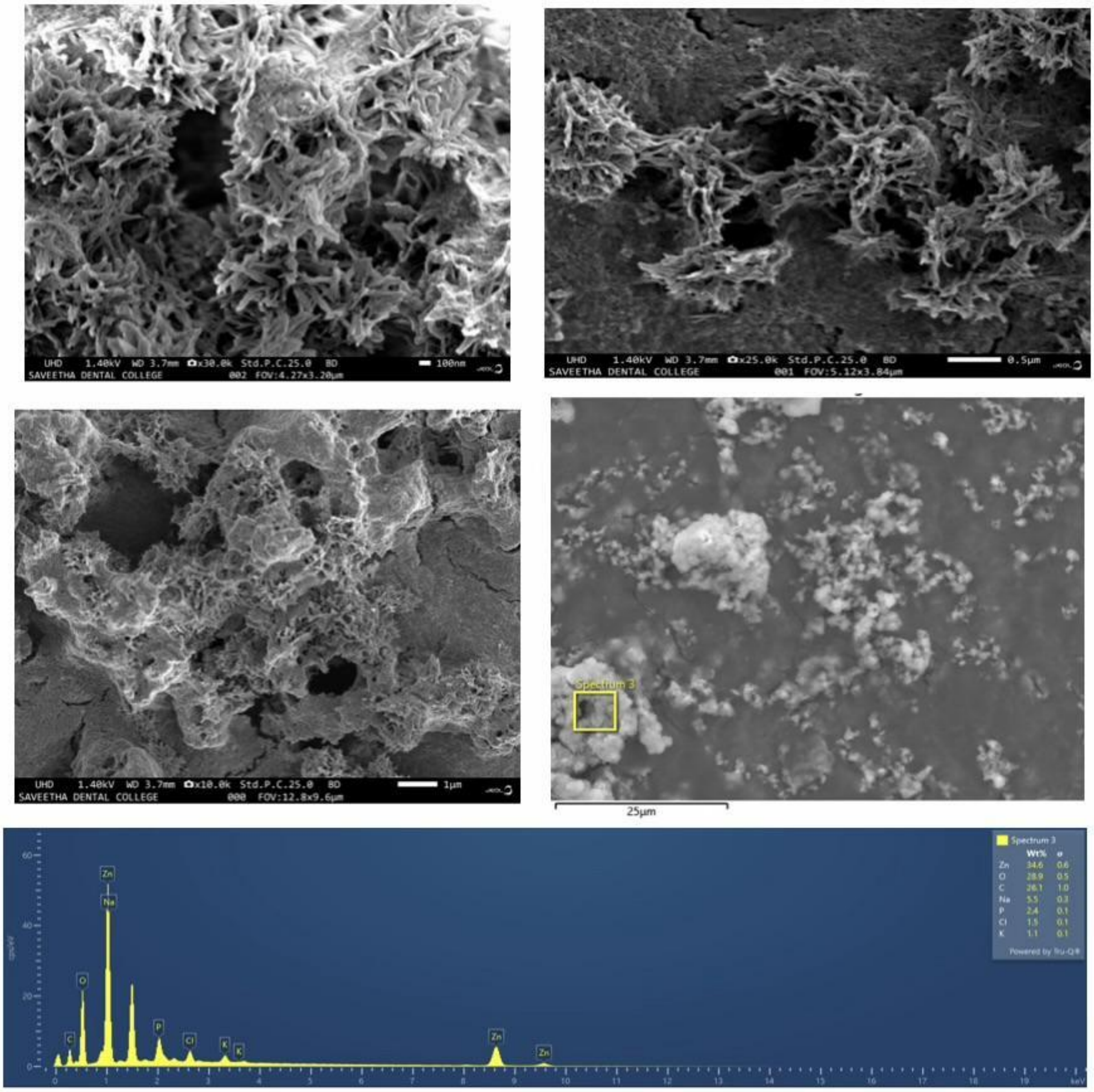
The developmental toxicity of the plant extract-mediated zinc oxide nanoparticle dental varnish was evaluated using zebrafish (*Danio rerio*) embryos exposed to different concentrations (1, 2, 4, 8, and 16 µL) from 24 to 96 hours post-fertilization (hpf). Embryonic development was assessed by recording the hatching rate, larval viability, mortality, and morphological changes.

The results demonstrated a concentration-dependent effect of the dental varnish on zebrafish embryo development. Embryos exposed to lower concentrations (1, 2, and 4 µL) showed normal embryonic development with high survival and hatching rates comparable to the control group. No significant developmental abnormalities or morphological defects were observed in these groups, indicating good biocompatibility at lower concentrations.

At higher concentrations (8 and 16 µL), a reduction in the hatching rate and larval viability was observed. These groups exhibited an increase in embryo mortality along with delayed hatching compared to the control group. Mild developmental abnormalities were also evident at the highest concentration, suggesting that toxicity increased with increasing concentration of the dental varnish.

Microscopic examination of the embryos throughout the exposure period showed progressive embryonic development in all groups, although embryos treated with higher concentrations displayed comparatively slower development. Representative stereomicroscopic images confirmed the differences in embryo morphology and hatching among the treatment groups.

Overall, the findings indicate that the green-synthesized zinc oxide nanoparticle dental varnish exhibited good biocompatibility at lower concentrations, while higher concentrations produced concentration-dependent developmental toxicity in the zebrafish embryo model. These results suggest that the formulated dental varnish has potential for dental applications when used within an optimal concentration range.



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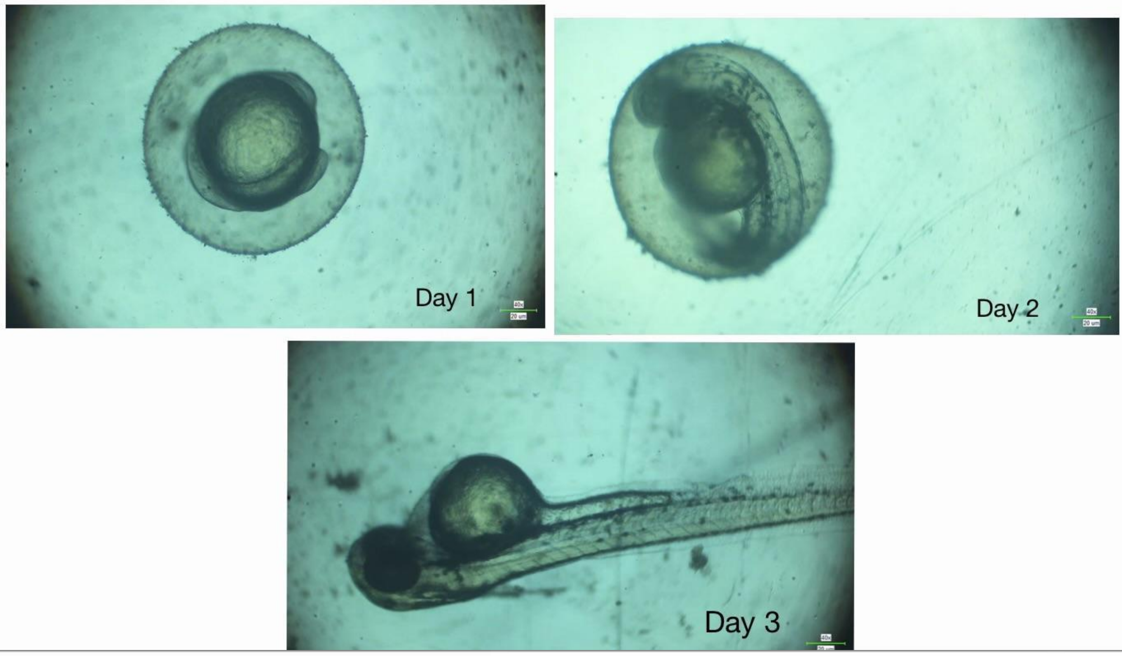
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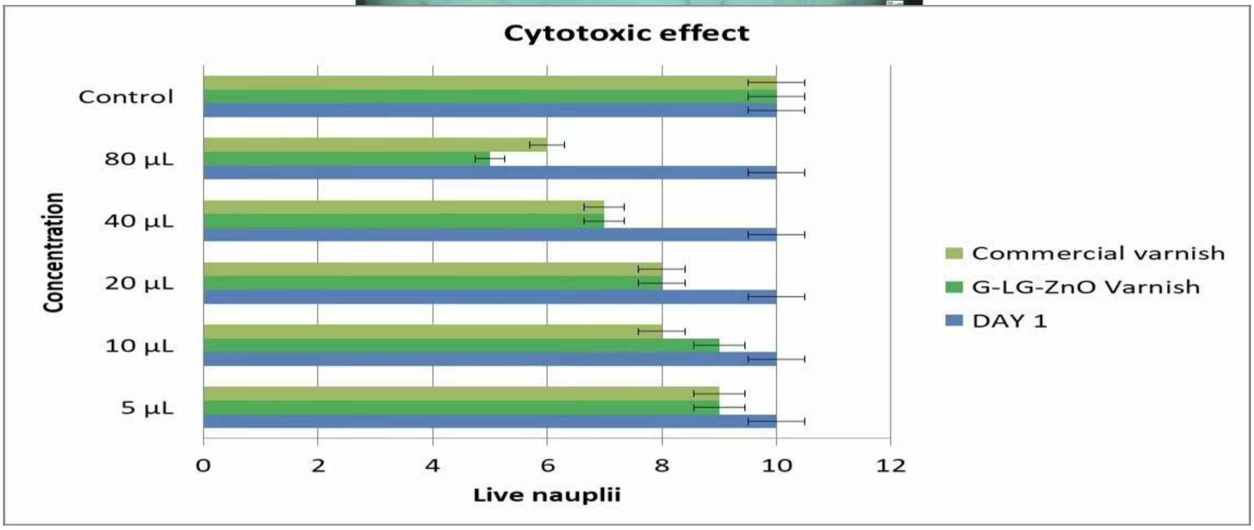
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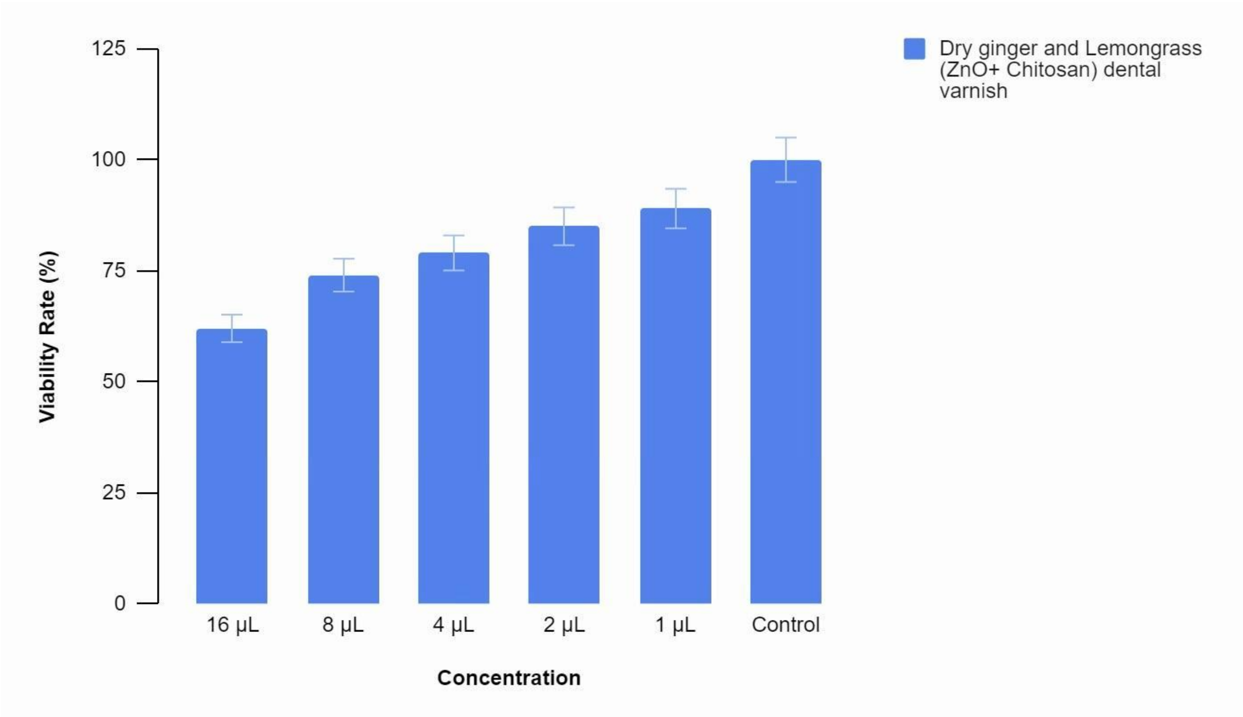

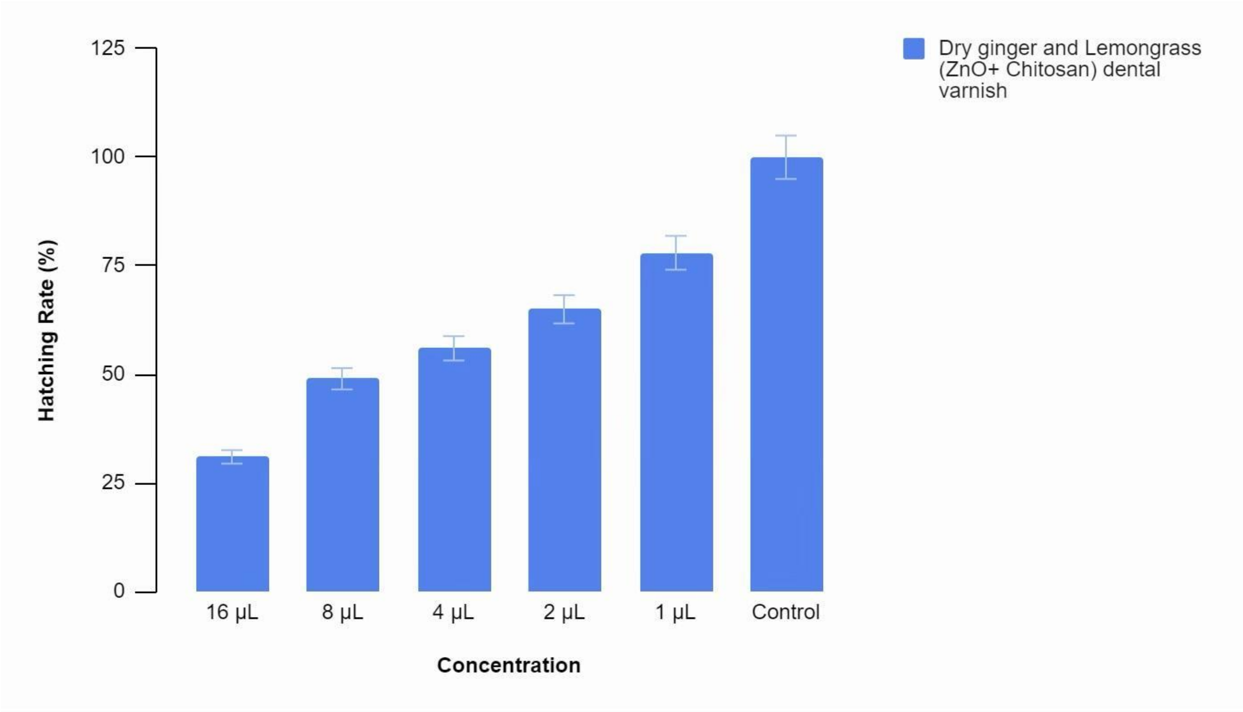

Discussion

The present study evaluated the developmental toxicity and biocompatibility of a green-synthesized zinc oxide nanoparticle (ZnO NP)-based dental varnish formulated using dry ginger (*Zingiber officinale*), lemongrass (*Cymbopogon citratus*), and chitosan polymer. The zebrafish (*Danio rerio*) embryo model was selected because of its sensitivity in detecting developmental toxicity and its established role in evaluating the safety of newly developed biomaterials. The results demonstrated that the formulated dental varnish exhibited good biocompatibility at lower concentrations, whereas higher concentrations produced concentration-dependent developmental toxicity, reflected by reduced hatching rate, decreased larval viability, and increased mortality.

Green synthesis has emerged as one of the most promising approaches for nanoparticle production because it employs biological materials such as plant extracts instead of hazardous chemical reducing agents. This eco-friendly method minimizes the use of toxic chemicals, reduces environmental pollution, lowers production costs, and improves the biological compatibility of nanoparticles. Plant extracts contain a wide variety of phytochemicals, including flavonoids, phenols, terpenoids, alkaloids, and tannins, which act as natural reducing and stabilizing agents during nanoparticle synthesis. Consequently, green-synthesized nanoparticles often demonstrate enhanced antimicrobial activity and reduced toxicity compared with chemically synthesized nanoparticles, making them highly suitable for biomedical and dental applications (1,2).

Zinc oxide nanoparticles have attracted considerable attention because of their broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria. Their antimicrobial effect is mainly attributed to the generation of reactive oxygen species, release of zinc ions, and disruption of bacterial cell membranes. These mechanisms contribute to inhibition of bacterial growth and prevention of biofilm formation, which are essential in controlling oral pathogens responsible for dental caries and periodontal diseases. Incorporation of ZnO nanoparticles into dental varnishes provides prolonged antimicrobial action while maintaining acceptable biocompatibility when used within an optimal concentration range (3, 4)

The use of medicinal plant extracts further enhances the biological properties of ZnO nanoparticles. Lemongrass contains citral and other phenolic compounds that exhibit potent antibacterial and antioxidant activities against oral microorganisms. Likewise, ginger is rich in gingerols and shogaols, which possess antimicrobial, anti-inflammatory, and antioxidant properties. These phytochemicals not only facilitate the green synthesis of nanoparticles but also contribute synergistically to their biological activity. The combination of ginger and lemongrass extracts with ZnO nanoparticles therefore represents a promising natural strategy for developing safer and more effective dental materials (5,6).

Chitosan was incorporated into the dental varnish because of its excellent biocompatibility, biodegradability, bioadhesive properties, and inherent antimicrobial activity. The positively charged amino groups of chitosan interact with negatively charged bacterial cell membranes, thereby inhibiting bacterial proliferation. In addition, chitosan improves the adhesion of dental varnishes to tooth surfaces and provides sustained release of the incorporated nanoparticles and phytochemicals, thereby prolonging their therapeutic effect. These characteristics make chitosan an ideal carrier for nanoparticle-based dental formulations (7).

The zebrafish embryo model has become an established vertebrate model for evaluating the safety of nanoparticles because of its transparent embryos, rapid embryonic development, high fecundity, and close genetic similarity to humans. Assessment of embryo survival, hatching rate, larval viability, and morphological development provides reliable evidence regarding developmental toxicity. In the present study, embryos exposed to lower concentrations of the formulated dental varnish developed normally without significant morphological abnormalities, indicating that the formulation was well tolerated. However, increasing concentrations resulted in delayed hatching and increased embryo mortality, suggesting that excessive nanoparticle exposure may interfere with normal embryonic development. Similar concentration-dependent toxicity has been reported in previous studies evaluating ZnO nanoparticles using zebrafish embryos (8, 9).

The observed reduction in hatching rate at higher concentrations may be attributed to oxidative stress induced by excessive nanoparticle accumulation around the embryo, resulting in delayed embryogenesis and impaired enzyme activity required for hatching. Likewise, increased mortality at higher concentrations could be associated with the generation of reactive oxygen species and disturbance of cellular homeostasis. Despite these findings, the absence of significant toxicity at lower concentrations suggests that the formulated dental varnish possesses a favorable safety profile for further investigation.

Overall, the findings of the present study indicate that the green-synthesized ZnO nanoparticle dental varnish formulated using ginger, lemongrass, and chitosan exhibits promising biocompatibility at lower concentrations while retaining the potential antimicrobial benefits of plant-mediated nanoparticles. Further studies involving physicochemical characterization, long-term cytotoxicity, antimicrobial efficacy against oral pathogens, and clinical evaluation are recommended to validate its safety and effectiveness before translation into routine dental practice.

Conclusion

The present study showed that the green-synthesized zinc oxide nanoparticle dental varnish prepared using dry ginger, lemongrass, and chitosan is a safe and biocompatible material at lower concentrations. The formulation supported normal embryonic development, good hatching, and high larval survival in the zebrafish embryo model. These findings suggest that the developed dental varnish has promising potential as an eco-friendly and effective material for preventive dental applications.

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