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Evaluation of Cytotoxicity and Embryotoxicity of Ocimum tenuiflorum-Ocimum gratissimum Mediated Silver Nanoparticle-Based Dental Varnish: A Comparative Study with Commercial Varnish

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Abstract **Background:** Silver nanoparticles (AgNPs) are widely recognized for their potent antimicrobial properties, making them promising candidates for dental applications. However, concerns regarding their potential cytotoxicity and embryotoxicity remain significant. This study evaluates the safety profile of a green-synthesized AgNPs-based dental varnish using *Ocimum tenuiflorum* and *Ocimum gratissimum* extracts as reducing and stabilizing agents. The study aims to compare its toxicity profile with a commercial dental varnish to assess its potential as a safer alternative in dental care. **Methods:** AgNPs were synthesized using *Ocimum tenuiflorum* and *Ocimum gratissimum* extracts, confirmed by visual color change and UV-Visible spectroscopy. Zebrafish embryos were selected as a model to evaluate toxicity. Embryos were exposed to different concentrations of the AgNPs-based dental varnish (5, 10, 20, 40 and 80 µg/mL), with their viability, hatching rates and morphological changes recorded. The commercial dental varnish was tested under identical conditions to serve as a comparison. **Results:** The AgNPs-based varnish displayed concentration-dependent toxicity. At lower concentrations (5 and 10 µg/mL), 100% viability and 100% hatching rates were observed, similar to the control. At 40 µg/mL, slight toxicity was evident, with viability decreasing to 85% and hatching rates reducing to 80%. At 80 µg/mL, the AgNPs varnish showed reduced viability (60%) and hatching rates (60%). In comparison, the commercial varnish exhibited greater toxicity, with only 50% hatching rates observed at 80 µg/mL and higher instances of morphological abnormalities in zebrafish embryos. **Conclusion:** The green-synthesized AgNPs-based dental varnish demonstrated reduced toxicity compared to the commercial dental varnish at higher concentrations. The antioxidant properties of phytochemicals present in *Ocimum* extracts, such as eugenol and rosmarinic acid, may have contributed to stabilizing the nanoparticles, thus minimizing oxidative stress. These findings suggest that the AgNPs-based varnish holds promise as a safer and eco-friendly dental care alternative. Further in vivo studies are recommended to assess its long-term safety and clinical efficacy.

Key Words Silver nanoparticles, *Ocimum tenuiflorum*, *Ocimum gratissimum*, dental varnish, zebrafish embryos, cytotoxicity, embryotoxicity, green synthesis, surface plasmon resonance

INTRODUCTION

The rising prevalence of dental caries and periodontal diseases has created a growing need for enhanced preventive treatments in dentistry. While fluoride varnishes have been widely adopted for their well-established efficacy in remineralizing enamel and reducing caries incidence, certain limitations necessitate the exploration of improved alternatives [1]. Fluoride varnishes primarily function by releasing fluoride ions over time, which enhance mineral uptake in demineralized enamel regions. However, concerns

such as aesthetic drawbacks, potential fluorosis risks and insufficient broad-spectrum antimicrobial efficacy have prompted researchers to explore innovative strategies to enhance dental materials for improved oral health outcomes [2,3].

Among emerging approaches, silver nanoparticles (AgNPs) have demonstrated significant potential due to their powerful antimicrobial properties, durability and capacity for sustained action. AgNPs are known for their unique physicochemical properties, including a high surface-to-volume ratio that enhances their ability to disrupt bacterial cell membranes and biofilms. These qualities have led to the incorporation of AgNPs into various dental products, including varnishes, to strengthen their protective potential [4].

In recent years, the green synthesis of AgNPs using plant-based reducing agents has gained popularity as an eco-friendly and biocompatible alternative to chemical synthesis methods. This method eliminates the use of harmful chemicals, thereby improving the safety profile of the resulting nanoparticles [5]. Notably, extracts from *Ocimum tenuiflorum* (Holy Basil) and *Ocimum gratissimum* (African Basil) have shown great promise as reducing and stabilizing agents in AgNP synthesis. Both plants are rich in bioactive compounds such as eugenol, flavonoids and phenolic acids, known for their antimicrobial, antioxidant and anti-inflammatory properties [6]. By leveraging these bioactive compounds during nanoparticle synthesis, researchers have successfully created AgNPs with enhanced stability, biocompatibility and antimicrobial potential-characteristics that are highly desirable in dental applications [7,8].

Dental varnishes are commonly used to provide a protective coating that releases active agents slowly over time, offering prolonged enamel protection and bacterial inhibition. While fluoride varnishes have remained the conventional choice, the incorporation of AgNPs synthesized via green methods offers an innovative solution that may overcome some of the limitations of traditional formulations [9]. Ocimum-mediated AgNPs not only provide effective antimicrobial action but also retain the therapeutic properties of the plant extracts, contributing additional protective benefits such as antioxidant effects and reduced inflammation [10]. These enhanced properties make Ocimum-mediated AgNP-based varnishes a promising alternative for sustained caries prevention, improved oral hygiene and increased patient comfort [11].

While AgNPs have demonstrated promising antibacterial potential, concerns remain regarding their potential cytotoxic and systemic effects. Dental varnishes, given their extended contact with oral tissues and possible ingestion, require thorough evaluation of their safety profiles. Cytotoxic studies are crucial to assess their impact on oral cells, while embryonic toxicology studies provide critical insights into the potential developmental effects of the varnish, particularly for vulnerable populations such as pediatric and prenatal patients [12,13].

This study aims to evaluate the cytotoxic and embryotoxic effects of a dental varnish formulated with *Ocimum tenuiflorum* and *Ocimum gratissimum*-mediated AgNPs. By comparing this formulation with a commercial dental varnish, the research aims to provide comprehensive insights into the safety and potential clinical applications of the Ocimum-mediated AgNP varnish. The findings are intended to contribute to the development of safe, biocompatible and sustainable dental materials for effective caries prevention and improved oral healthcare.

MATERIALS AND METHODS

Preparation of Ocimum Tenuiflorum and Ocimum Gratissimum Based Herbal Formulation

To prepare a herbal formulation, 1 g of Ocimum tenuiflorum and 1 g of *Ocimum gratissimum* were precisely added to 100 mL of distilled water. The solution was heated using a heating mantle at 60 degrees Celsius for 15-20 minutes. After boiling, the mixture was slowly filtered through filter paper. The filtrate, which held the extract, was then stored for future nanoparticle synthesis.

Synthesis of Ocimumtenuiflorum and Ocimum Grattissimum Herbal Formulation Mediated Silver Nanoparticles and its Based Dental Varnish

For the green synthesis of silver nanoparticles (AgNPs), a 1 mM silver nitrate solution was prepared by dissolving silver nitrate in 80 mL of distilled water. Subsequently, 20 mL of the filtered herbal extract formulation was added to the silver nitrate solution. The resulting mixture was centrifuged at 8000 rpm for 10 minutes to facilitate nanoparticle formation and separation. The pellet obtained post-centrifugation was collected and stored for subsequent use in dental varnish preparation.

To formulate the dental varnish incorporating Ocimum tenuiflorum and Ocimum gratissimum based AgNPs, 7 mL of a chitosan solution was combined with 2.5 mL of ethanol and 500 µL of the synthesized AgNPs. The mixture was thoroughly blended using a vortex mixer for 1-2 hours to ensure a homogeneous distribution of AgNPs throughout the varnish matrix.

Cytotoxic Effect- Brine Shrimp Lethality Assay

The cytotoxicity of the AgNPs based dental varnish, formulated with *Ocimum tenuiflorum* and Ocimum grattissimum herbal formulation, was tested through the Brine Shrimp Lethality Assay. *Artemia salina* nauplii, which were hatched from cysts in synthetic seawater, were used as the test organisms. Varying concentrations of the oral rinse (5, 10, 20, 40 and 80 µg/mL) were created by diluting the

stock solution in seawater. A control group containing only seawater, without any herbal oral rinse, served as the reference for baseline comparison.

For each concentration, 10 nauplii were placed in separate vials containing 5 mL of the respective test solution. These vials were maintained under constant light exposure and observed at intervals of 24 and 48 hours. The survival of the nauplii was recorded at each time point and the mortality rate was determined by comparing the number of dead nauplii in each test vial to the control group.

Embryonic Toxicology Evaluation

To evaluate the acute cytotoxicity of *Ocimum tenuiflorum* and *Ocimum gratissimum* mediated silver nanoparticles (AgNPs)-based dental varnish, we conducted a zebrafish embryonic toxicology study. Wild-type zebrafish (*Danio rerio*) were sourced from local vendors in India and maintained under controlled conditions, with a stable temperature of 28±2°C, a 14:10-hour light/dark cycle and a pH range between 6.8 and 8.5. The fish were fed a nutritious diet of dry blood worms or optimal feed twice daily to promote healthy growth and reproduction. Fertilized eggs were collected by pairing one female with three males in a breeding tank, with the viable eggs carefully rinsed in E3 medium to remove any residues before use in the experiment.

The zebrafish embryos were then transferred to culture plates with varying well sizes (6, 12 and 24 wells), placing 20 embryos in each well with 2 mL of the respective treatment solution. Embryos were exposed to different concentrations of the *Ocimum*-mediated AgNP-based dental varnish, alongside a commercial dental varnish for comparison. Treatment concentrations ranged from 5 to 80 µg/L, with untreated control groups included to monitor baseline development. Each treatment and control group was replicated three times to ensure reliable results. The treated plates were covered with foil to prevent light interference and maintained at a constant temperature of 28°C. Every 12 hours, dead embryos were removed to maintain culture conditions.

Throughout the 24-78 hour post-fertilization period, the zebrafish embryos were observed using a stereo microscope to track developmental progress and to record any mortality, hatching rates and abnormal morphology. Observations focused on identifying any potential dose-dependent toxicity effects of the AgNP-based varnish, particularly in higher concentrations. The percentage of embryo/hatchling mortality was documented every 24 hours and hatching rates were recorded to identify any developmental delays or deviations. Any abnormalities, including spinal deformities, yolk sac edema, or craniofacial malformations, were also noted. Images of malformed embryos were taken using a COSLAB-Model: HL-10A light microscope and the percentage of abnormal embryos was documented daily to provide a comprehensive overview of potential toxicological effects.

Statistical Analysis

The data collected from the brine shrimp lethality assay and zebrafish embryo hatching and viability rate experiments were expressed as mean ± standard deviation (SD). Statistical significance between different concentrations and groups was determined using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for multiple comparisons. A p-value of less than 0.05 ($p<0.05$) was considered statistically significant. All statistical analyses were performed using GraphPad Prism software (version 8.0.1).

RESULT AND DISCUSSION

Visual observation of green synthesized AgNPs

The biosynthesis of silver nanoparticles (AgNPs) was conducted using silver nitrate as a precursor, with *Ocimum tenuiflorum* and *Ocimum gratissimum* based herbal formulation acting as reducing agent. The progression of nanoparticle synthesis was visually monitored through observable color changes in the reaction mixture.

Upon the initial addition of silver nitrate to the herbal formulation, the solution exhibited a pale yellow hue, indicating the starting condition of the reaction mixture. After 1 hour of incubation, the solution began to show a noticeable change to a light brown color (Figure 1), suggesting the early formation of AgNPs. This color shift is characteristic of the surface plasmon resonance (SPR) of silver nanoparticles, indicating the initial stages of nanoparticle nucleation.

After 48 hours, the solution underwent a further transformation, with the color intensifying to a dark brown shade (Figure 2), indicative of complete reduction of silver ions (Ag+) to silver nanoparticles (Ag0). The deepening of the color corresponds to the increased concentration and size of AgNPs as the reaction reached completion.

The change in color from pale yellow to dark brown strongly supports the successful synthesis of AgNPs mediated by the phytochemicals present in *Ocimum tenuiflorum* and *Ocimum gratissimum* extracts.

UV-Visible Spectroscopy of AgNPs

The formation of silver nanoparticles (AgNPs) was further confirmed and monitored using UV-Visible spectroscopy by measuring the Surface Plasmon Resonance (SPR) peaks at specific time intervals. The SPR is a characteristic feature of metal nanoparticles, with silver nanoparticles typically exhibiting a peak in the range of 400-450 nm.

In this study, the UV-Visible absorption spectra of the *Ocimum tenuiflorum* and *Ocimum gratissimum*-mediated AgNPs were recorded at intervals of 1 hour, 24 hours, 36 hours and 48 hours. At 1 hour, a small and broad peak around 430 nm was observed, indicating the initial nucleation and formation of AgNPs. As the reaction proceeded, the intensity of the SPR peak increased progressively, which suggests an increase in the concentration of nanoparticles.

At 24 hours, the SPR peak at 430 nm became more defined, indicating the significant growth and stabilization of

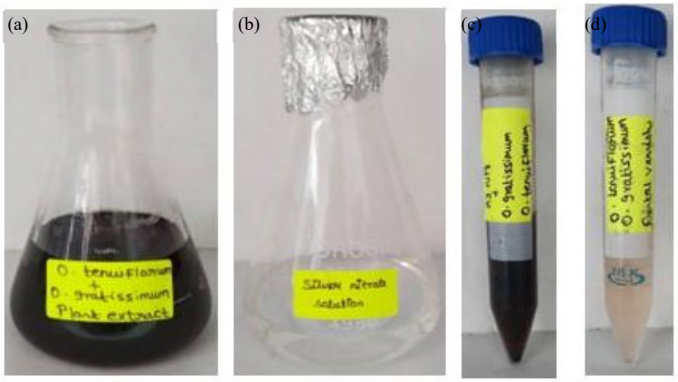

Figure 1(a-d): Synthesis of Herbal Formulation mediated Silver nanoparticles and its based dental varnish, (a) Herbal formulation, (b) Silver nitrate as precursor, (c) AgNPs pellet and (d) AgNPs based dental varnish

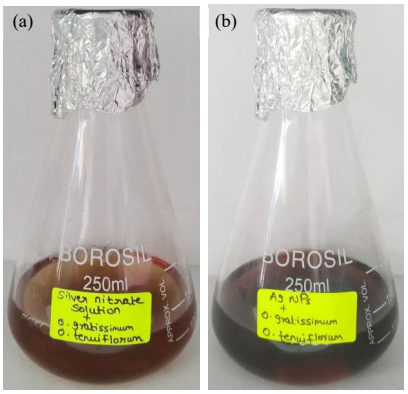

Figure 2(a-c): Visual observation of green synthesized AgNPs, (a) 1 hr initial colour change and (b) 48 hr final colour change

AgNPs. By 36 hours, the peak maintained its position but showed a further increase in intensity, confirming the continued formation and stabilization of AgNPs.

Finally, after 48 hours, the SPR peak at 430 nm was fully developed, with maximum absorbance, indicating that the synthesis process had reached its saturation point. The stable SPR peak confirms the successful and complete reduction of Ag+ ions to AgNPs mediated by the bioactive compounds in *Ocimum tenuiflorum* and *Ocimum gratissimum*.

The cytotoxic potential of the *Ocimum tenuiflorum* and *Ocimum gratissimum*-mediated silver nanoparticles (AgNPs) incorporated into dental varnish was assessed using the Brine Shrimp Lethality Assay. The assay tested various concentrations of the AgNPs-based dental varnish (5, 10, 20, 40 and 80 µg/mL) at two time points-Day 1 and Day 2-by measuring the percentage of live nauplii.

As shown in Figure 3, the 5 µg/mL concentration exhibited minimal toxicity, with 95-100% of the brine shrimp nauplii surviving at both Day 1 and Day 2, indicating that this concentration of AgNPs-based dental varnish is safe for biological systems. Similar results were observed at 10 µg/mL and 20 µg/mL, where 85-100% of the nauplii remained alive, demonstrating that the lower concentrations of the dental varnish showed no significant cytotoxic effects.

At 40 µg/mL, a slight reduction in the survival rate was observed, with about 75-80% of the nauplii surviving on both days. However, at the highest concentration of 80 µg/mL, the nauplii survival dropped significantly, particularly on Day 2, with only 20% of live nauplii. This clearly suggests that while the dental varnish is relatively safe at lower concentrations, it becomes increasingly cytotoxic at higher doses.

The control group, without the dental varnish, exhibited 100% survival over the two days, confirming the assay's reliability. These results suggest a concentration-dependent cytotoxic effect of the AgNPs-based dental varnish, with potential safety at lower concentrations but a need for caution at higher dosages.

Embryonic Toxicology

Zebrafish Embryonic Toxicological Evaluation

The effect of AgNPs-based dental varnish on zebrafish (*Danio rerio*) embryonic development was assessed by monitoring the hatching rate at different concentrations of the varnish (5, 10, 20, 40 and 80 µg/mL). The hatching rate was recorded at 96 hours post-fertilization, with the results presented in Figure 3.

The 5 µg/mL concentration exhibited a 100% hatching rate, comparable to the control group, indicating no adverse

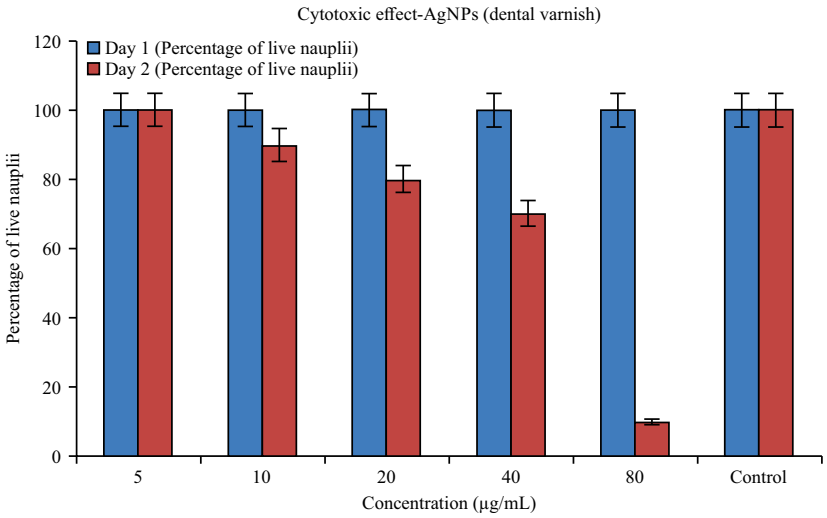

Figure 3: Cytotoxic effect of AgNPs based dental varnish tested with brine shrimp nauplii

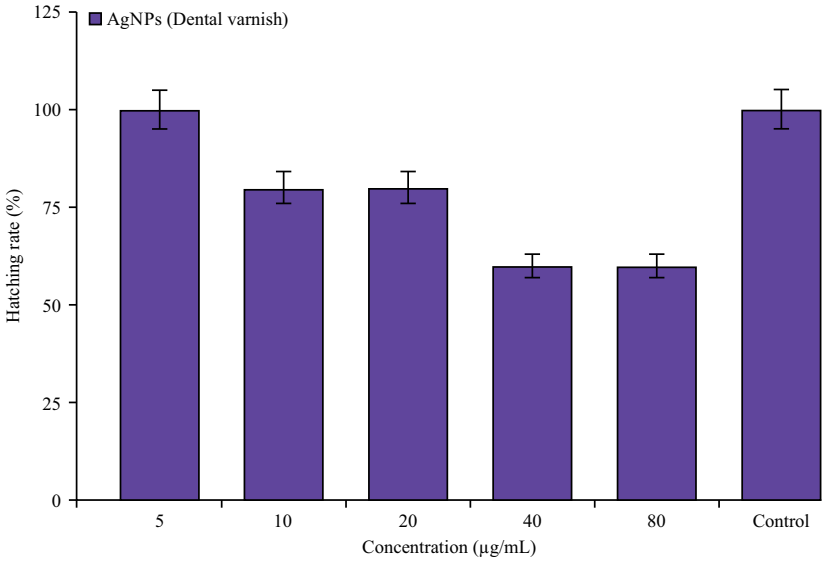

Figure 4: Hatching Rate of Zebrafish Embryos Exposed to *Ocimum tenuiflorum* and *Ocimum gratissimum* mediated AgNPs based dental varnish

effects of the AgNPs-based dental varnish at this concentration. A slight decrease in hatching rate was observed at 10 µg/mL and 20 µg/mL, where both concentrations resulted in a 80% hatching rate, suggesting a minor impact on embryonic development.

At higher concentrations, particularly at 40 µg/mL and 80 µg/mL, the hatching rate dropped significantly to 60%, demonstrating a clear concentration-dependent toxicological effect on the embryos. Despite this reduction, the hatching rate remained above 50%, indicating moderate embryonic viability at these higher concentrations.

The control group, which was not exposed to the AgNPs-based dental varnish, maintained a 100% hatching rate, further validating the assay's reliability. These findings suggest that the AgNPs-based dental varnish has a minimal impact on zebrafish embryonic development at lower concentrations, but at higher concentrations, it may impede normal hatching processes.

The impact of AgNPs-based dental varnish on zebrafish embryonic viability was assessed by calculating the viability rate at varying concentrations of AgNPs (5, 10, 20, 40 and 80 µg/mL) after 96 hours of exposure. The results are summarized in Figure 4.

At the lowest concentrations of 5 µg/mL and 10 µg/mL, the viability rate remained at 100%, which is identical to the control group, indicating that the AgNPs-based dental

varnish has no cytotoxic effect on zebrafish embryos at these concentrations. This suggests that the varnish is biocompatible at these lower doses.

At 20 µg/mL, the viability rate decreased to 80%, marking the first observable reduction in embryo viability. A similar viability rate of 80% was also recorded at 40 µg/mL, indicating a moderate level of cytotoxicity at these concentrations.

The highest concentration of 80 µg/mL exhibited the most significant reduction in viability, with only 60% of the embryos remaining viable. This confirms a concentration-dependent cytotoxic effect of the AgNPs-based dental varnish.

The control group, which was not exposed to the dental varnish, consistently exhibited a 100% viability rate, reinforcing the conclusion that the cytotoxicity observed is due to the AgNPs in the varnish.

These results demonstrate that while the AgNPs-based dental varnish is non-toxic at lower concentrations, higher concentrations can adversely affect the viability of zebrafish embryos, showing a clear dose-dependent response.

Zebrafish Embryonic Hatching Rate Evaluation for Commercial Varnish

To assess the effects of commercial varnish on zebrafish embryonic development, hatching rates were measured at various concentrations (5, 10, 20, 40 and 80 µg/mL) and compared to a control group. The results are presented in Figure 5.

At 5 µg/mL, the commercial varnish exhibited a hatching rate of approximately 100%, which was similar to the control group. This indicates that at lower concentrations, the commercial varnish does not significantly affect the hatching process. However, as the concentration increased, a reduction in hatching rate was observed. At 10 µg/mL, the hatching rate dropped slightly to 80% and at 20 µg/mL, it remained consistent at around 80%.

At higher concentrations, the impact became more pronounced. The hatching rate dropped to 60% at 40 µg/mL and 50% at 80 µg/mL, indicating a concentration-dependent inhibition of the hatching process. Despite this, the control group, which was not exposed to the varnish, maintained a 100% hatching rate, confirming that the observed effects were due to the commercial varnish exposure.

These findings suggest that while the commercial varnish has minimal effects at lower concentrations, it exhibits significant inhibitory effects on the hatching of zebrafish embryos at higher concentrations, highlighting the potential for developmental toxicity depending on exposure levels.

Zebrafish Embryonic Viability Rate Evaluation for Commercial Varnish

The effect of commercial varnish on zebrafish embryo viability was evaluated at different concentrations (5, 10, 20, 40 and 80 µg/mL). The viability rate was measured 96 hours post-fertilization, with the results shown in Figure 6.

At the lowest concentration of 5 µg/mL, the viability rate remained at 100%, indicating no adverse effects on embryonic viability. This trend continued at 10 µg/mL, where the viability rate was also 100%, suggesting that at these lower concentrations, the commercial varnish is non-toxic to zebrafish embryos.

However, at 20 µg/mL, the viability rate dropped slightly to 80%, indicating a moderate level of cytotoxicity at this concentration. A similar viability rate of 80% was observed at 40 µg/mL, showing a consistent moderate reduction in embryo viability.

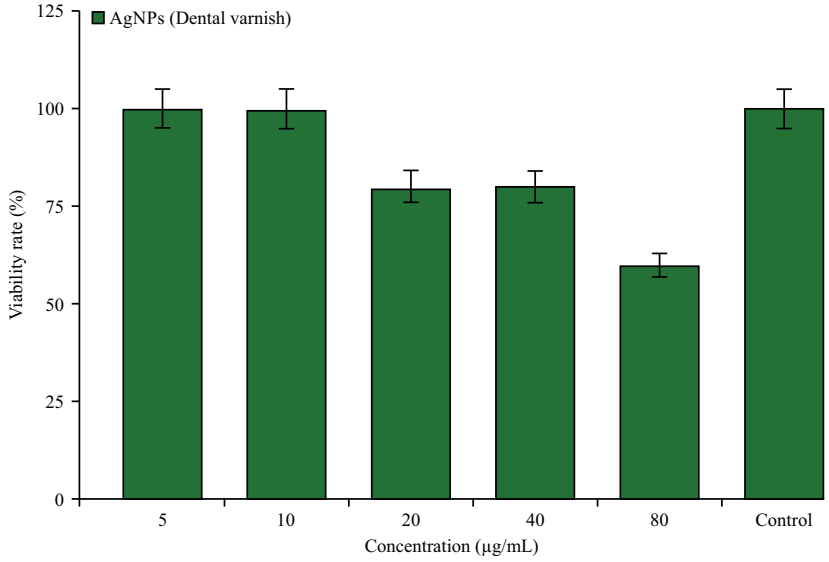

Figure 5: Viability rate of zebrafish embryos exposed to *Ocimum tenuiflorum* and *Ocimum gratissimum* mediated AgNPs based dental varnish

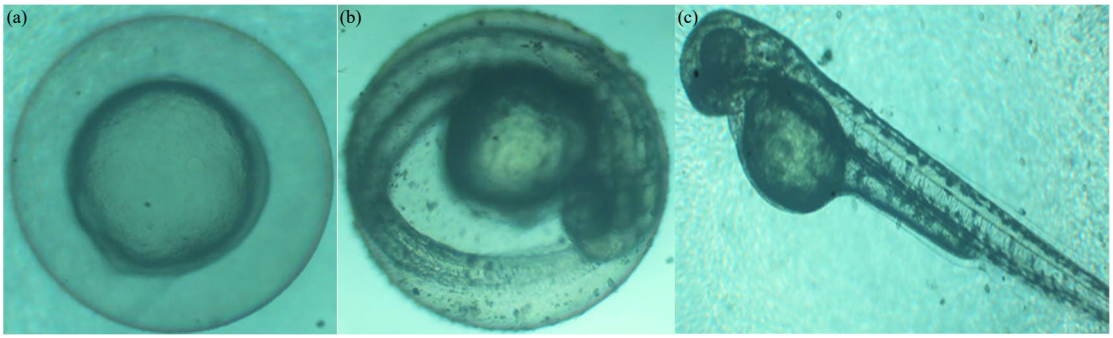

Figure 6(a-c): Zebrafish embryos treated with AgNPs based dental varnish, (a) Day 1, (b) Day 2 and (c) Day 3

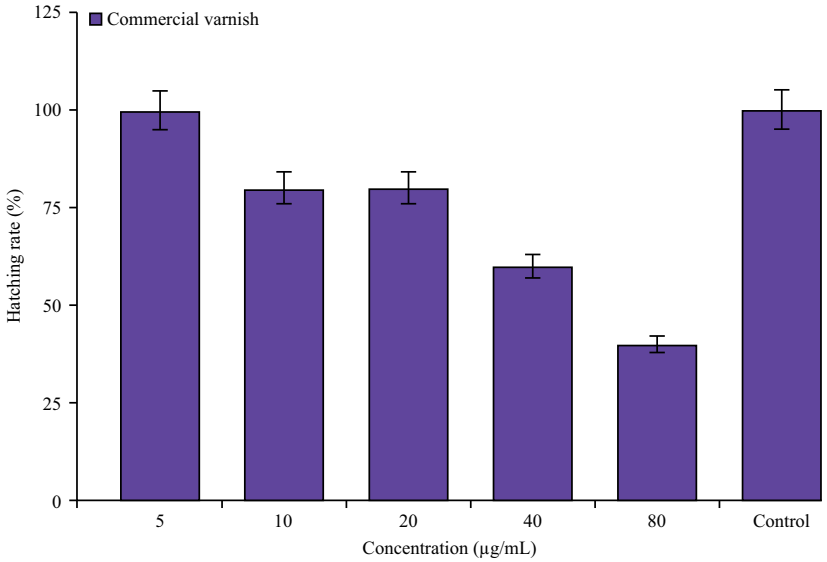

Figure 7: Hatching Rate of Zebrafish Embryos Exposed to commercial dental varnish

At the highest concentration of 80 µg/mL, the viability rate further declined to 60%, indicating a significant cytotoxic effect at this concentration. This demonstrates a clear dose-dependent decrease in viability as the concentration of the commercial varnish increases.

The control group, which was not exposed to the commercial varnish, maintained a 100% viability rate, validating the reliability of the assay. These results suggest that while the commercial varnish is non-toxic at lower concentrations, it can adversely affect zebrafish embryonic viability at higher concentrations, indicating potential developmental toxicity

Comparative Analysis of AgNPs-Based Dental Varnish and Commercial Varnish

The effects of both green synthesized AgNPs-based dental varnish and commercial varnish on zebrafish embryonic hatching rate and viability rate were evaluated and a concentration-dependent trend was observed in both varnishes. At 5 µg/mL, both varnishes exhibited a 100% hatching rate, indicating no adverse effects on the hatching process at lower concentrations. This trend continued at 10 µg/mL, where both varnishes showed a slight decrease to 80% hatching rate, with no significant difference between them. At 20 µg/mL, the hatching rate remained steady at 80% for both varnishes. However, at 40 µg/mL, the hatching rate dropped to 60% for both, demonstrating that both varnishes exhibited a similar inhibitory effect on the hatching process at moderate concentrations. Notably, at 80 µg/mL, the AgNPs-based dental varnish demonstrated a 60% hatching rate, while the commercial varnish showed a slightly lower 50% hatching rate, suggesting that the AgNPs-based varnish may exhibit slightly lower developmental toxicity at higher concentrations (Figure 7).

In terms of viability rate, the AgNPs-based dental varnish and the commercial varnish also showed similar trends across different concentrations. Both varnishes maintained a 100% viability rate at 5 µg/mL and 10 µg/mL, indicating no cytotoxic effects at lower concentrations. At 20 µg/mL, a reduction in the viability rate to 80% was observed for both varnishes, suggesting moderate cytotoxicity. This trend continued at 40 µg/mL, with both varnishes again showing an

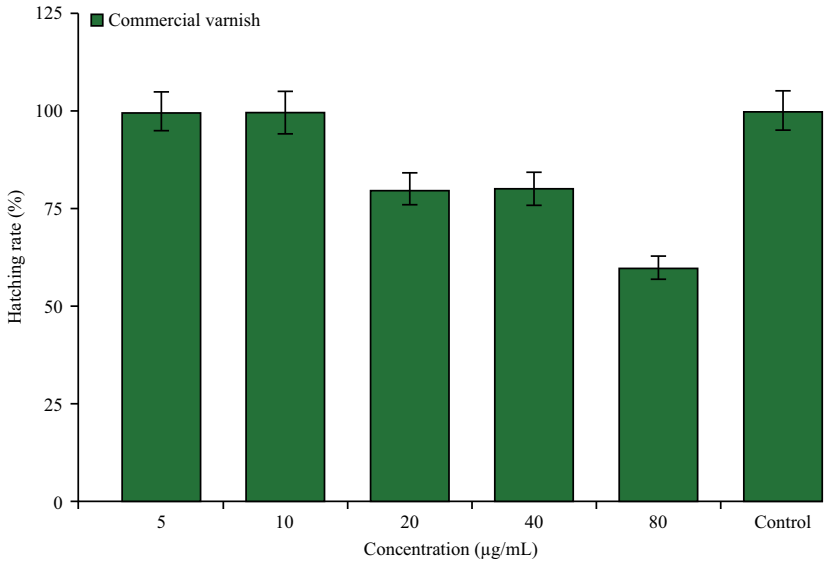

Figure 8: Viability Rate of Zebrafish Embryos Exposed to commercial dental varnish

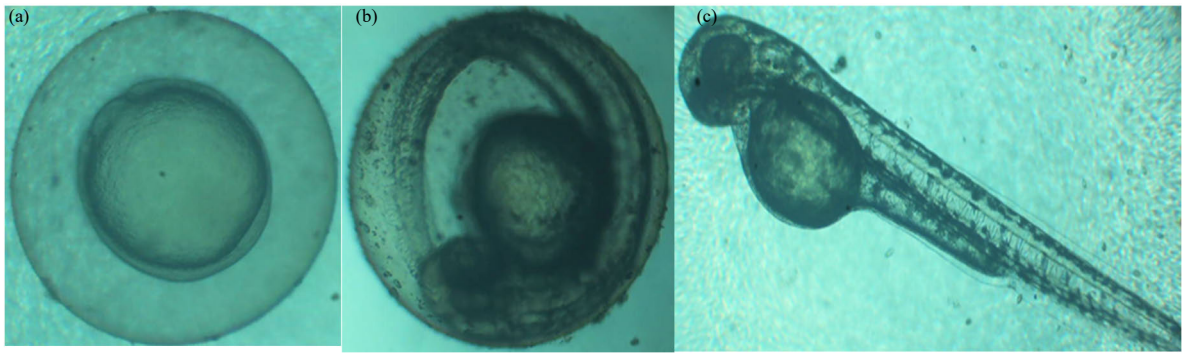

Figure 9(a-c): Zebrafish embryos treated with commercial dental varnish, (a) Day 1, (b) Day 2 and (c) Day 3

80% viability rate. At the highest concentration of 80 µg/mL, both varnishes demonstrated a significant decrease in viability, with both resulting in a 60% viability rate, indicating a comparable level of cytotoxicity at this concentration (Figure 8, 9).

Overall, both the AgNPs-based dental varnish and the commercial varnish demonstrated comparable impacts on zebrafish embryonic development, particularly in terms of hatching and viability rates. At lower concentrations, both varnishes were non-toxic, maintaining high hatching and viability rates. However, at higher concentrations, a concentration-dependent reduction in hatching and viability rates was observed for both. While the AgNPs-based dental varnish showed a slightly higher hatching rate at the highest concentration, indicating potentially lower developmental toxicity compared to the commercial varnish, the overall cytotoxicity of both varnishes was similar, especially with regard to embryo viability. These results suggest that both products exhibit comparable effects, with only slight advantages for the AgNPs-based varnish at higher concentrations.

DISCUSSION

This study evaluated the cytotoxic and embryotoxic effects of a dental varnish formulated using *Ocimum tenuiflorum* and *Ocimum gratissimum*-mediated silver nanoparticles (AgNPs) and compared its toxicity profile with that of a commercially available dental varnish. AgNPs are recognized for their strong antimicrobial properties; however, understanding their potential toxicological impact is crucial to ensuring safe clinical application, particularly at higher concentrations.

The successful synthesis of AgNPs using herbal extracts was confirmed through visual observation and UV-visible spectroscopy. The observed color change from pale yellow to light brown within one hour indicated the initial formation of

AgNPs. The color intensified to dark brown after 48 hours, confirming the completion of the reduction process. This color shift is attributed to the excitation of surface plasmon resonance (SPR), a distinctive optical property linked to AgNP formation [14].

UV-visible spectroscopy further confirmed AgNP synthesis by detecting an SPR peak at around 430 nm. The increasing peak intensity over 48 hours indicated stable nanoparticle formation and uniform particle size distribution, supporting the effective synthesis of AgNPs using *Ocimum tenuiflorum* and *Ocimum gratissimum* extracts [15,16].

The cytotoxic assessment of the AgNPs-based varnish revealed concentration-dependent toxicity. At lower concentrations (5 and 10 µg/mL), the varnish maintained 100% viability, similar to the control group. However, as concentrations increased (20, 40 and 80 µg/mL), a progressive reduction in viability was noted, with rates declining to 80% at 20 and 40 µg/mL and further to 60% at 80 µg/mL. This reduction aligns with the known release of silver ions from nanoparticles, which can induce oxidative stress, trigger apoptosis and impair cellular function [17].

Similarly, the AgNPs-based varnish showed a concentration-dependent reduction in zebrafish embryo hatching rates. While the varnish exhibited 100% hatching at 5 and 10 µg/mL, the hatching rate decreased to 80% at 20 and 40 µg/mL and further to 60% at 80 µg/mL. This embryotoxic effect is consistent with the known interference of silver ions in developmental processes, which can impair cell division and disrupt growth mechanisms [18].

Comparing the AgNPs-based varnish with the commercial dental varnish revealed similar trends in toxicity. Both varnishes maintained 100% viability and hatching rates at lower concentrations. However, at 80 µg/mL, the AgNPs-based varnish demonstrated slightly reduced toxicity, with a 60% hatching rate compared to 50% for the commercial varnish. This reduced toxicity may be attributed to the presence of phytochemicals such as eugenol and rosmarinic acid in *Ocimum tenuiflorum* and *Ocimum gratissimum*, which possess antioxidant properties that mitigate oxidative stress and stabilize the nanoparticles [19,20].

The cytotoxic and embryotoxic effects observed at higher concentrations may be linked to oxidative stress induced by reactive oxygen species (ROS) generated by the release of silver ions. ROS are known to cause DNA damage, mitochondrial dysfunction and apoptosis, contributing to cell death and impaired development [9]. Additionally, AgNPs are reported to cross the blood-brain barrier, posing a potential neurotoxic risk. However, the protective role of phytochemicals in the Ocimum extracts may have counteracted these effects to some extent, reducing overall toxicity [21].

Future Scope

Although this study provides valuable insights into the cytotoxic and embryotoxic effects of AgNPs-based varnish, further investigations are warranted to determine its long-term safety and clinical efficacy. Future research should focus on chronic exposure studies to assess the accumulation of silver ions in tissues and their potential effects on systemic health. Long-term studies involving mammalian models will be crucial to better understand the impact of AgNPs-based varnishes in realistic clinical scenarios.

Moreover, optimizing the nanoparticle formulation to achieve improved biocompatibility without compromising antimicrobial efficacy is essential. Exploring different particle sizes, surface coatings and concentrations may help enhance safety while maintaining the desired therapeutic effects. Comparative studies with alternative nanoparticles such as zinc oxide (ZnO) or titanium dioxide (TiO) may also reveal superior formulations that strike a balance between efficacy and minimal toxicity. Further investigation into the molecular mechanisms of AgNP-induced toxicity can provide valuable insights into developing safer dental materials [22,23].

CONCLUSION

The dental varnish formulated using *Ocimum tenuiflorum* and *Ocimum gratissimum*-mediated silver nanoparticles exhibited lower toxicity compared to a commercially available dental varnish, particularly at higher concentrations. The synthesis of AgNPs was confirmed through visual observation and UV-visible spectroscopy, with stable SPR peaks indicating successful nanoparticle formation. While the AgNPs-based varnish demonstrated minimal toxicity at lower concentrations, a dose-dependent increase in cytotoxicity and embryotoxicity was observed at higher concentrations.

The slight advantage observed in reduced developmental toxicity with the Ocimum-mediated AgNPs-based varnish may be attributed to the antioxidant effects of bioactive compounds such as eugenol and rosmarinic acid, which help mitigate oxidative stress. These findings suggest that AgNPs-based varnishes may serve as a safer alternative to commercial dental varnishes if appropriately formulated. Future studies should prioritize optimizing nanoparticle concentrations, conducting long-term safety assessments and exploring potential clinical applications for improved caries prevention and oral health management.

Limitations

Several limitations of this study must be acknowledged. The zebrafish embryo model, while widely accepted for toxicity testing, may not fully replicate human biology. Thus, further in vivo studies involving mammalian models are necessary for more comprehensive safety assessments. Additionally, the tested concentration range may not capture all clinically relevant scenarios. Exploring both lower and higher concentration ranges would provide a broader understanding of the toxicity profile.

Furthermore, this study only evaluated the short-term effects (up to 96 hours) of AgNPs exposure. Future research

should examine long-term outcomes, especially given that dental varnishes may remain in the oral cavity for extended periods. Studies assessing prolonged exposure, cumulative toxicity and potential interactions with oral tissues will be crucial in establishing the long-term safety of AgNPs-based dental varnishes.

Ethical Considerations

This study was conducted following ethical guidelines for in vitro and embryotoxicity research. Approval was obtained from the Institutional Ethical Committee. All experiments involving zebrafish embryos were performed in accordance with the OECD guidelines for fish embryo toxicity testing to ensure humane and ethical treatment. Standard protocols were followed to minimize stress and ensure proper care of the test organisms throughout the study.

Conflict of Interest

The authors declare no conflict of interest related to this study. The research was conducted independently without any financial or commercial influence that could compromise the integrity of the findings.

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