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Green Synthesis of Selenium Nanoparticles and Evaluation of Cytotoxic and Embryonic Toxicity in Dental Varnish Incorporating *Ziziphus oenoplia*

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Abstract

The utilization of *Ziziphus oenoplia* and selenium nanoparticles in dental varnish formulations presents a novel approach to enhancing antimicrobial and antioxidant properties. This study aimed to synthesize eco-friendly selenium nanoparticles and evaluate the cytotoxic and embryological toxic effects of a dental varnish composed of these nanoparticles and *Ziziphus oenoplia*. The plant extract was prepared using *Ziziphus oenoplia*, and selenium nanoparticles were synthesized with sodium selenate and distilled water. The cytotoxic effects were assessed using the Brine Shrimp Lethality Assay. Results indicated that a concentration of 10 µg/ml did not impact the viability and hatching rate of live nauplii. However, increasing the concentration to 20-40 µg/ml negatively affected both the hatching rate and viability. This study underscores the potential of incorporating *Ziziphus oenoplia* into dental varnish, while also highlighting the need for further research to evaluate the toxic effects of selenium nanoparticles as a component of dental varnish.

Keywords: *Ziziphus oenoplia*, selenium nanoparticles, dental varnish, cytotoxicity, embryological toxicity, Brine Shrimp Lethality Assay, antimicrobial properties, antioxidant properties.

1 Introduction

In recent years, the field of dentistry has undergone significant transformations, driven by the increasing emphasis on patient safety and the need to address environmental concerns(Soraya, Najoua et al. , Ambika, Manojkumar et al. 2019). The adoption of biocompatible and environmentally friendly materials has emerged as a pivotal focus within the profession. This shift is largely influenced by the recognition of the adverse impacts associated with conventional materials and the growing awareness of sustainability issues(Kishore, Priya et al. 2020, Chojnacka, Moustakas et al. 2023). Among the innovative solutions addressing these

challenges is nanotechnology, which offers promising advantages in enhancing the quality and performance of dental materials. Selenium nanoparticles (SeNPs) have garnered particular interest due to their unique physicochemical properties and potential applications in oral care.Selenium, a mineral known for its impressive biological activity, exhibits significant antioxidant properties, making it highly suitable for dental applications. SeNPs are characterized by their distinct electrical properties and large surface area-to-volume ratio, which enhance their efficacy in various dental applications(Rieshy, PRIYA et al. 2020, Tayyeb, Priya et al. 2024). These nanoparticles have been explored for their potential in medication delivery systems, antibacterial elements, and teeth remineralization products(Roshan, Jothipriya et al. 2020, Marunganathan, Kumar et al. 2024). Additionally, the anti-inflammatory and antioxidant effects of selenium make it desirable for addressing dental issues such as caries and periodontal diseases.The synthesis of nanoparticles is a crucial aspect in the development of new materials, particularly when considering sustainability and environmental impact(Senthil, Sundaram et al. 2022, Velumani, Arasu et al. 2023). Traditional synthesis methods often involve hazardous materials and conditions, posing risks to both the environment and human health. In contrast, green synthesis methods offer an environmentally friendly alternative by utilizing biocompatible materials and reagents. This approach not only minimizes ecological footprints but also enhances the safety profile of the synthesized nanoparticles. A promising avenue for the green synthesis of SeNPs is the utilization of *Ziziphus oenoplia*, a medicinal plant rich in bioactive compounds(Ravikumar, Marunganathan et al. 2024).*Ziziphus oenoplia*, also known as *Lannea coromandelica*, is native to various regions in Asia and Africa and belongs to the Rhamnaceae family. This plant has been extensively used in traditional medicine due to its diverse pharmacological properties. It exhibits antioxidant, antimicrobial, and anti-inflammatory activities, and plays a role in wound healing processes. The high content of flavonoids, tannins, saponins, and phenolic compounds in *Ziziphus oenoplia* makes it an excellent candidate for green synthesis. These phytochemicals effectively reduce selenium ions into nanoparticles and impart additional medicinal properties to the final nanomaterial(Nasim, Kumar et al. 2020, Ponmanickam, Gowsalya et al. 2022) .The use of *Ziziphus oenoplia* extract as a reducing and stabilizing agent for SeNP synthesis not only provides environmental benefits by avoiding toxic chemicals but also leverages the healing properties of the plant. This approach aligns with the principles of green chemistry, emphasizing sustainability and safety. The incorporation of medicinal plants in nanoparticle synthesis aims to produce SeNPs that are compatible with the oral health system, thereby enhancing their potential for dental treatments(Rajeshkumar and Lakshmi 2021).

The present study aims to synthesize selenium nanoparticles using *Ziziphus oenoplia* extract and evaluate their application in dental varnish formulations. The synthesized SeNPs will be characterized to determine their physicochemical properties(Anbu, Boomiga et al. 2022, Umapathy, Pan et al. 2024). Furthermore, the study will investigate the antibacterial properties and biocompatibility of the dental varnish incorporating SeNPs. A critical aspect of this research is the evaluation of cytotoxic and embryonic toxicology of the synthesized SeNPs to ensure their safety for oral applications.The synthesis process will involve the preparation of *Ziziphus oenoplia* extract, which will be used to reduce selenium ions to form nanoparticles. The characterization of SeNPs will be performed using techniques such as UV-Vis spectroscopy, transmission electron microscopy (TEM), and dynamic light scattering (DLS). These techniques will provide insights into the size, morphology, and stability of the synthesized nanoparticles(Mahapatra, Mohanta et al. 2011).Following the synthesis and

characterization, the potential application of SeNPs in dental varnish formulations will be explored. The antibacterial properties of the dental varnish will be assessed through in vitro studies against common oral pathogens. Additionally, the cytotoxicity of the dental varnish will be evaluated using cell viability assays to determine its safety for oral tissues(Shoeb, Mir et al. 2005, Anbarasu, Vinitha et al. 2024).

The embryonic toxicology studies will be conducted using model organisms to assess the impact of SeNPs on developmental processes. These studies are essential to ensure that the dental varnish does not pose any risks to developing tissues or organisms, thereby confirming its safety for use in pregnant patients or during the early stages of life.In conclusion, this study aims to synthesize environmentally friendly selenium nanoparticles using *Ziziphus oenoplia* extract and evaluate their potential application in dental varnish formulations. By leveraging the unique properties of SeNPs and the medicinal benefits of *Ziziphus oenoplia*, this research seeks to develop biocompatible dental materials that address both oral health and environmental sustainability. The findings of this study will contribute to the growing body of knowledge on green synthesis methods and their applications in dentistry, paving the way for safer and more effective dental treatments(Rajeshkumar, Lakshmi et al. 2021, Saravanan and Sundaram 2022).

2 Materials and methods

2.1 Preparation of plant extract

To prepare the plant extract, 1 gram of *Ziziphus oenoplia* plant powder was mixed with 100 ml of distilled water in a clean, heat-resistant container. The mixture was then placed on a heating mantle and heated at a controlled temperature of approximately 80-90°C for 15-20 minutes to facilitate the extraction of the bioactive compounds. This heating process ensures better solubilization of the phytochemicals present in the plant material. After the heating period, the mixture was allowed to cool to room temperature to prevent degradation of heat-sensitive compounds. Once cooled, the extract was filtered using Whatman filter paper No. 1 to remove any residual plant debris and obtain a clear filtrate. The filtration process involved pouring the mixture through the filter paper placed in a funnel, allowing the liquid to pass through while retaining the solid particles. The resultant clear filtrate was collected in a sterile container and used as the plant extract for further experiments. This extract served as the reducing and stabilizing agent for the green synthesis of selenium nanoparticles. The prepared plant extract was then stored at 4°C to maintain its stability and prevent microbial contamination until it was used for nanoparticle synthesis(Shyamala and Manikandan 2019, Khalid, Martin et al. 2024).

2.2 Preparation of nanoparticles

For the preparation of selenium nanoparticles, 0.034 grams of sodium selenate was dissolved in 70 ml of distilled water, ensuring complete dissolution. Subsequently, this sodium selenate solution was combined with 30 ml of *Ziziphus oenoplia* extract. The mixture was then placed in an orbital shaker and agitated continuously for 48 hours to facilitate the synthesis of selenium nanoparticles. After the incubation period, the synthesized nanoparticles were subjected to UV-Vis spectroscopy to confirm their formation. Following confirmation, the nanoparticle solution

was centrifuged at 8000 rpm for 10 minutes to separate the nanoparticles. The resultant pellets, containing the selenium nanoparticles, were carefully collected and stored for further biomedical applications(Mohanasundari, Anbalagan et al. 2022).

2.3 Fish maintenance and SeNPs exposure

Wild-type zebrafish (Danio rerio) were acquired from local Indian vendors and were housed in individual tanks under controlled conditions of temperature ($28^0 \pm 2^0$C), light/darkcycle (14:10 h), and pH (6.8–8.5). The fishes were fed with commercially available dry blood worms or optimum food twice daily. Zebrafish embryos were obtained by crossing one female and three males per breeding tank, and viable eggs were collected and rinsed at least three times with freshly prepared E3 medium without methylene blue. The study involved the placement of fertilized eggs in culture plates of varying well sizes (6, 12, and 24 wells) with 20 embryos per 2 mL solution per well. The experimental treatment and control groups were replicated three times. To prepare the experimental treatment, a stock suspension of SeNPswith five different concentrations was freshly made and added directly to the E3 medium. The solution was sonicated for 15 minutes to disperse the nanoparticles while maintaining a pH range of 7.2-7.3. Healthy fertilized embryos were exposed to different concentrations of SeNPs ranging from (5, 10, 20, 40, and 80 µg/mL)for 24 to 96 hours post fertilization. The SeNPs were added to the E3 medium where the embryos were incubated. Control groups were also included in the experiment. Dead embryos were removed from the nanoparticles exposed groups every 12 hours. All experimental plates were wrapped in foil to exclude light and maintained at 28°C(Johnson, Shanmugam et al. 2022, Raj, Martin et al. 2024).

2.4 Zebrafish embryo evaluation

Throughout the exposure period following fertilization, the developmental stages of Zebrafish embryos were monitored using a stereo microscope. The embryos were subjected to various concentrations of selenium nanoparticles (5, 10, 20, 40, and 80 µg/mL) for 24-78 hpf. Embryonic mortality and hatching rates were assessed at 24-hour intervals. The study endpoints included embryo/hatchling mortality, hatching rate, and the identification and documentation of any malformations among the embryos and larvae in both control and treatment groups. Photographs of malformed embryos were captured using a COSLAB - Model: HL-10A light microscope and the percentage of abnormal embryos was recorded every 24 hours(Duraisamy, Ganapathy et al. 2021).

2.5 Brine shrimp lethality assay

Two grams of iodine-free salt were weighed and dissolved in 200 ml of distilled water to prepare a saline solution. Six well ELISA plates were then prepared by filling each well with 10-12 ml of the saline solution. Ten nauplii were carefully added to each well, and selenium nanoparticles were added to achieve concentration levels of 5, 10, 20, 40, and 80 µg/mL. The plates were incubated for 24 hours to allow interaction between the nauplii and the selenium nanoparticles. After the incubation period, the number of live nauplii in each well was recorded. The percentage of dead nauplii was calculated using the formula:

(Number of dead nauplii / (Number of dead nauplii + Number of live nauplii)) × 100.

3. Results

3.1 Preparation of plant extract

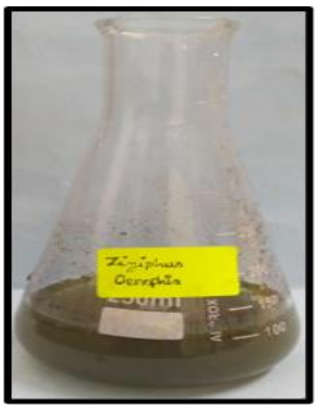

Figure 1 **Figure 2**

Fig 1 Plant extract powder
Fig 2Plant extract in distilled water

3.2 Preparation of nanoparticles

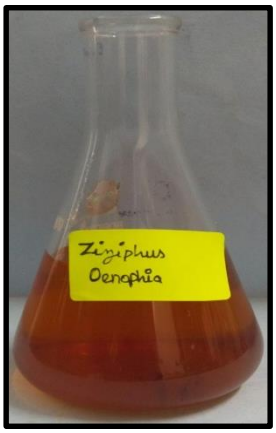

Figure 3 **Figure 4** **Figure 5**

Fig 3 plant extract
Fig 4 Final extract of*Ziziphus oenoplia*
Fig 5 SeNPs

3.3 Cytotoxic Effects

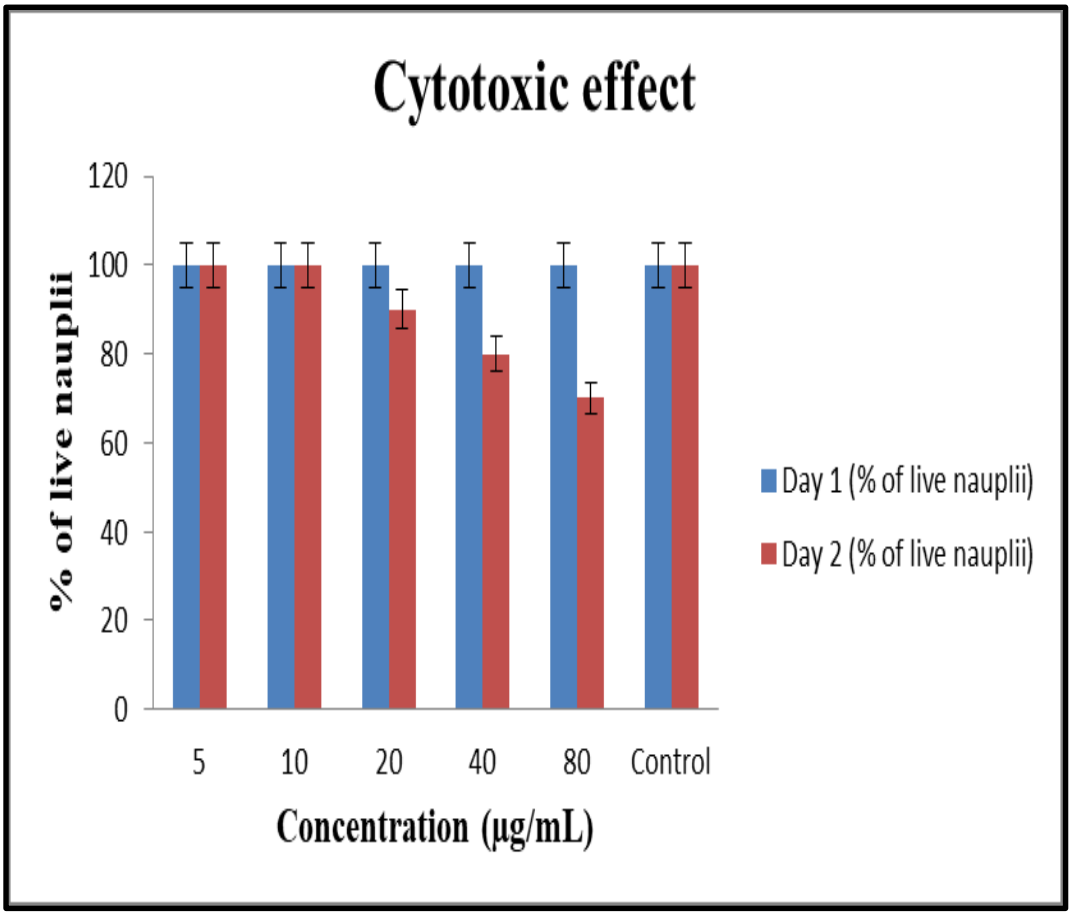

Figure 6: Figure 6 demonstrates a concentration-dependent decrease in the percentage of live nauplii on day 2, indicating cytotoxic effects of *Ziziphus oenoplia* extract at higher concentrations.

In the cytotoxicity assessment, the results indicated that at a concentration of 5 µg/ml, the percentage of live nauplii remained unaffected at 100% on both day 1 and day 2. Similarly, at 10 µg/ml, the live nauplii exhibited no adverse effects on either day, maintaining a 100% survival rate. However, at a concentration of 20 µg/ml, a decrease in the percentage of live nauplii was observed only on day 2, dropping to 90%, while day 1 showed no impact. At 40 µg/ml, the live nauplii were unaffected on day 1 but exhibited a significant decrease in survival rate on day 2, falling to 80%. This trend continued at 80 µg/ml, where a significant decrease to 70% in the percentage of live nauplii was noted on day 2, indicating a concentration-dependent cytotoxic effect. Throughout the experiment, the control group showed no impact on the percentage of live nauplii, confirming the specific cytotoxic effects of the selenium nanoparticles(Chokkattu, Mary et al. 2022).

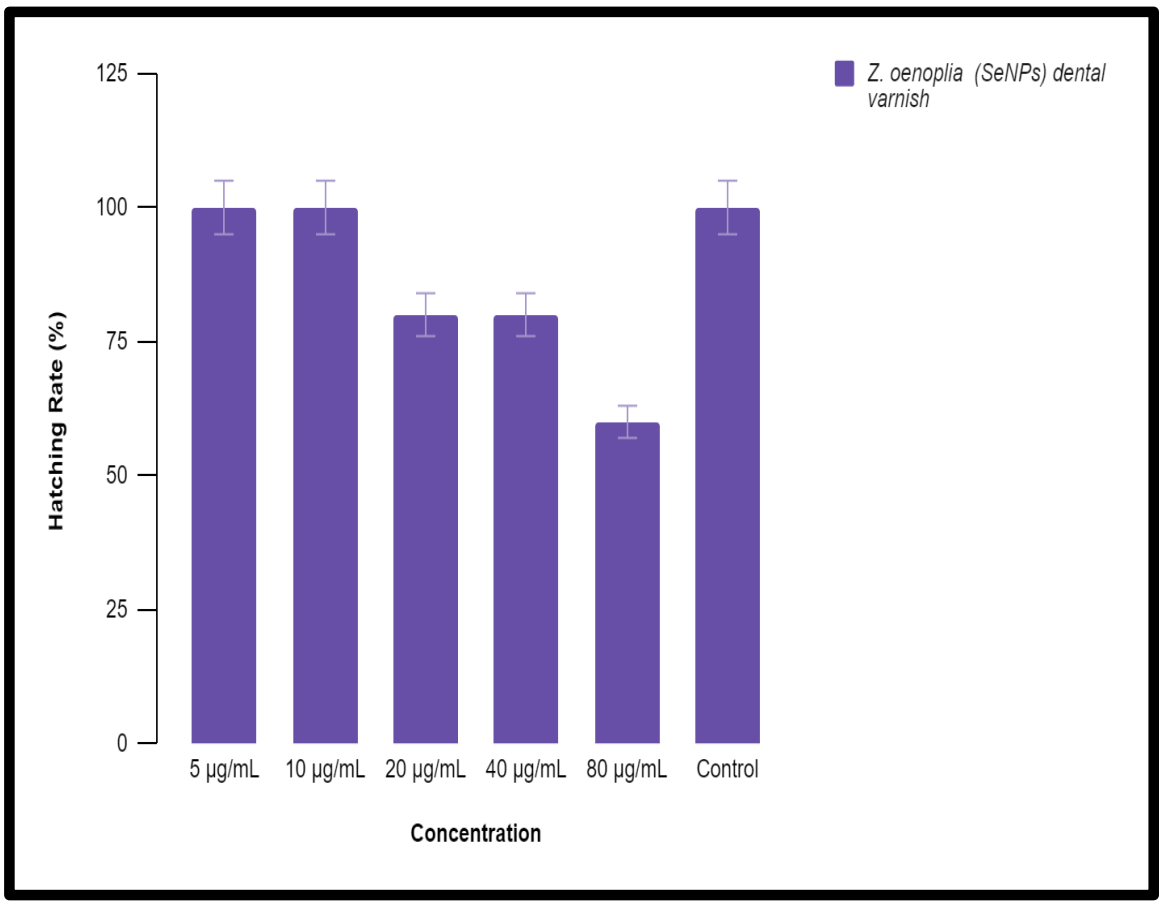

Figure 7: Figure illustrates a significant decrease in hatching rate as the concentration increases from 20 to 80 µg/ml

In Figure 7, the hatching rate analysis revealed distinct responses to varying concentrations of the tested substance. At concentrations of 5 µg/ml and 10 µg/ml, the hatching rate remained unaffected, maintaining a consistent rate. However, as the concentration increased beyond 10 µg/ml, noticeable effects on hatching rate became apparent. Specifically, at 20 µg/ml and 40 µg/ml, the hatching rates were similar, approximately at 75%. Further increasing the concentration to 80 µg/ml resulted in a decreased hatching rate, ranging between 50% and 75%. In contrast, the control group showed no significant deviation from a hatching rate of 100%, indicating no adverse effects under normal conditions. These findings underscore the concentration-dependent impact of the substance on the hatching process, with higher concentrations correlating with decreased hatching rates observed after the incubation period(Arjun, Sangeetha et al. , Duraisamy, Ganapathy et al. 2021).

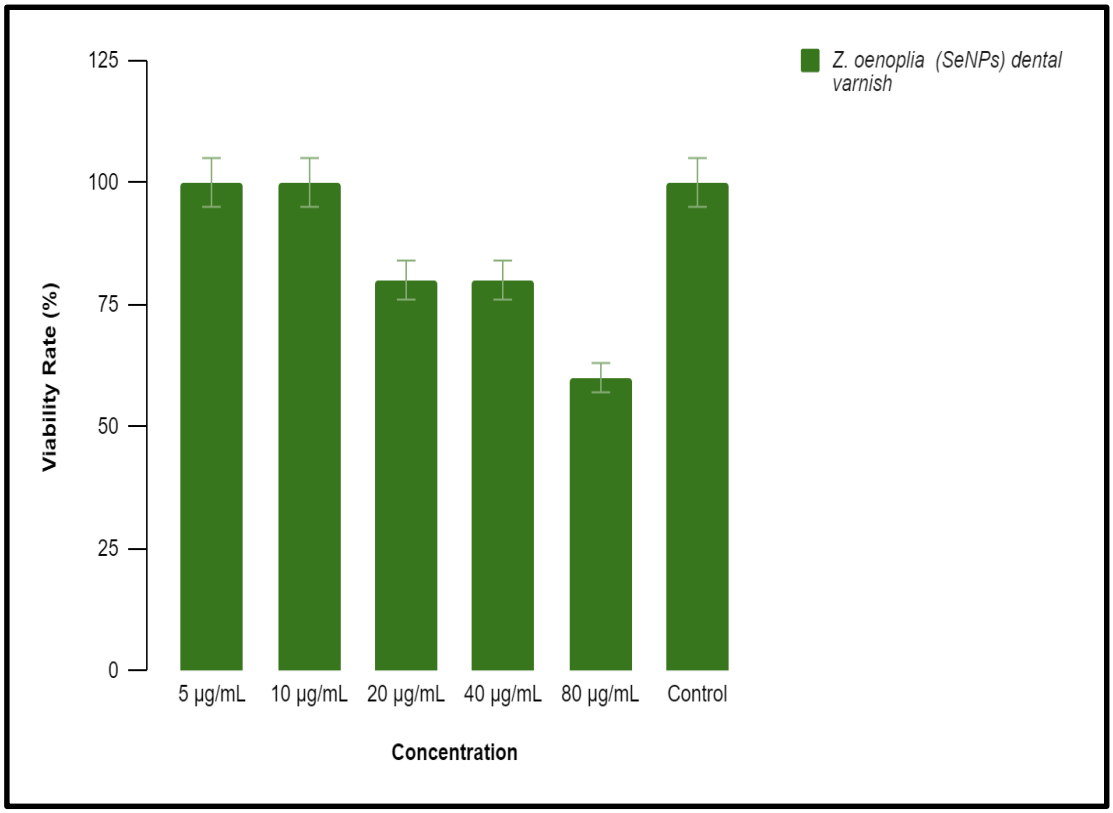

Figure 8: Figure demonstrates a decrease in viability rate as concentration increases from 20 to 80 µg/ml

In Figure 8, which depicts the viability rate, a pattern similar to that observed in the hatching rate graph is evident. At lower concentrations, specifically 5 µg/ml and 10 µg/ml, the viability rate remained unaffected, maintaining a robust survival rate of 100%. However, as the concentration of the substance increased to 20 µg/ml and 40 µg/ml, a noticeable decline in viability rate was observed, stabilizing at approximately 75% across both concentrations. Further escalation to 80 µg/ml resulted in a significant decrease in viability rate, dropping to slightly above 50%. Throughout the experiment, the control group exhibited no deviations in viability rate, remaining consistently unaffected. These findings reinforce the concentration-dependent impact of the substance on viability, with higher concentrations correlating with decreased viability rates, highlighting potential cytotoxic effects at elevated levels.

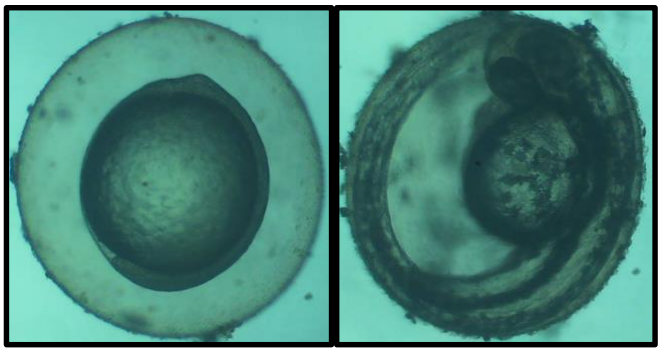

Figure 9 **Figure 10**

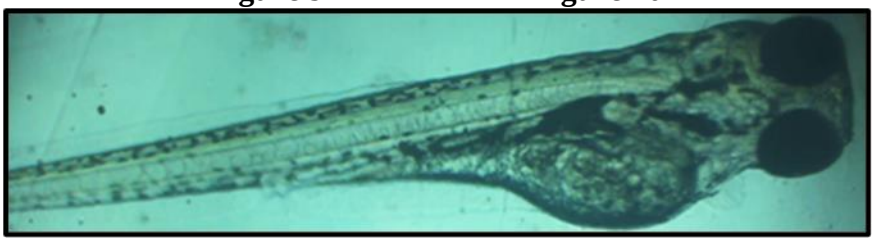

Figure 11

Fig 9 Day 1 - Live fertilized nauplii egg
Fig 10 Day 2 - hatched egg
Fig 11 Day 3 - hatched larva

The study aimed to synthesize selenium nanoparticles (SeNPs) using Ziziphus-mediated green synthesis and assess their impact on zebrafish embryos, focusing on cytotoxic and embryological toxicity effects. The findings indicate that concentrations up to 10 µg/ml had no discernible effect on nauplii hatching and viability rates. However, at concentrations ranging from 20 to 80 µg/ml, there was a clear negative impact observed, with significant decreases in both hatching and viability rates. These results highlight the potential of Ziziphus-mediated green synthesis to produce biocompatible SeNPs that could enhance the functional properties of dental varnishes, particularly in enhancing antibacterial efficacy against microorganisms on dental surfaces. Future research endeavors could delve deeper into understanding the mechanisms underlying these observed toxic effects, potentially uncovering pathways that could further optimize the application of SeNPs in dental treatments for improved patient outcomes and safety(NivedaRajeshwaran and Rajeshkumar 2021).

4 Discussion

In this study, we explored the synthesis of selenium nanoparticles (SeNPs) using *Ziziphus oenoplia* extract and evaluated their potential application in dental varnish formulations(Nahrin, Junaid et al. 2022). The utilization of SeNPs in dentistry holds promise due to their unique physicochemical properties and biological activities, particularly as antimicrobial and antioxidant agents. The green synthesis method employed, utilizing *Ziziphus oenoplia* as a reducing and stabilizing agent, aligns with current trends towards sustainable and eco-friendly nanoparticle synthesis methods(Prathap , Thirugnanasampandan, Ramya et al. 2017).

The results from the cytotoxicity assessment using the Brine Shrimp Lethality Assay indicated that lower concentrations of SeNPs (10 µg/ml) did not significantly impact the viability or

hatching rate of live nauplii. This suggests that at lower doses, SeNPs synthesized with *Ziziphus oenoplia* extract exhibit minimal toxicity, highlighting their potential safety for biomedical applications. However, as the concentration of SeNPs increased to 20-40 µg/ml, there was a notable decrease in both hatching rate and viability, indicating a concentration-dependent cytotoxic effect. This finding underscores the importance of optimizing nanoparticle concentrations to ensure biocompatibility and minimize adverse effects in potential dental applications.The observed antimicrobial properties of SeNPs are particularly promising for dental varnish formulations aimed at preventing microbial growth on dental surfaces. Selenium's ability to inhibit bacterial growth, coupled with its antioxidant properties, could contribute significantly to improving oral health outcomes, including the prevention of caries and periodontal diseases. Moreover, the use of *Ziziphus oenoplia* in nanoparticle synthesis adds an additional layer of bioactivity, potentially enhancing the therapeutic efficacy of SeNPs in dental care(Nivetha, Sakthi et al. 2020, Bupesh, Saravanan et al. 2022).

It is important to note the environmental and health benefits associated with green synthesis methods. Traditional nanoparticle synthesis processes often involve hazardous chemicals and energy-intensive procedures, contributing to environmental pollution and posing risks to human health. In contrast, green synthesis methods using plant extracts offer a sustainable alternative by utilizing natural, biocompatible materials and reducing environmental impact. This approach aligns with global efforts towards sustainable development and supports the shift towards greener technologies in various industrial sectors, including dentistry.

Future research directions could focus on further elucidating the mechanisms underlying the cytotoxic effects of SeNPs at higher concentrations and exploring strategies to mitigate these effects while maintaining their therapeutic efficacy. Additionally, investigating the long-term effects of SeNP-containing dental varnishes on oral tissues and microbial communities would provide valuable insights into their safety and effectiveness in clinical settings. Moreover, exploring the potential synergistic effects of SeNPs with other bioactive compounds from *Ziziphus oenoplia* could lead to the development of enhanced dental materials with multifunctional properties(Suksamrarn, Suwannapoch et al. 2005).

5 Conclusion

In conclusion, this study demonstrates the feasibility of synthesizing selenium nanoparticles using *Ziziphus oenoplia* extract and highlights their potential application in dental varnish formulations. The cytotoxicity assessment revealed concentration-dependent effects on viability and hatching rates of nauplii, suggesting careful consideration of nanoparticle concentrations in dental applications. The incorporation of *Ziziphus oenoplia* extract in green synthesis offers a sustainable approach to producing biocompatible SeNPs with enhanced antimicrobial and antioxidant properties. Further research is warranted to fully understand the mechanisms underlying SeNP toxicity and to optimize their formulation for safe and effective use in dental care.

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