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Green synthesis of selenium nanoparticles using *Azadirachta indica* extract and its antimicrobial activity and embryonic toxicology evaluation

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Abstract

The study aimed to assess the antimicrobial activity of *Azidirachta indica* mediated selenium nanoparticles (SeNPs) against oral pathogens and investigate their embryotoxic effects using zebrafish embryos. The concentrations of SeNPs tested were 25 µg/mL, 50 µg/mL, and 100 µg/mL, with comparisons made to a standard antimicrobial agent. The green synthesized SeNPs were characterized using UV-Visible spectrophotometer. The antimicrobial activity was evaluated against oral pathogens including C. albicans, E. coli, S. mutans, and Lactobacillus sp. The hatching rates and viability of zebrafish embryos were assessed at different concentrations of SeNPs. The green synthesized SeNPs showed its absorption peak at 360nm. SeNPs exhibited varying degrees of antimicrobial activity against the tested pathogens, with the highest inhibition observed at 100 µg/mL for C. albicans, E. coli, and S. mutans. The hatching and viability rates of zebrafish embryos decreased with increasing concentrations of SeNPs, indicating a dose-dependent action. The results suggest that A. indica mediated SeNPs possess significant antimicrobial activity against oral pathogens, with potential as an antimicrobial agent. However, the embryotoxicity study revealed a concentration dependent impact on hatching success and viability of zebrafish embryos at higher concentrations of SeNPs. A. indica mediated SeNPs show promise as antimicrobial agents against oral pathogens, but their embryotoxic effects warrant further investigation to determine safe concentrations for potential applications.

Keywords: Green synthesis, Azadirachta indica, Selenium nanoparticles, Antimicrobial agent, Embryotoxicity

Introduction

Selenium nanoparticles (SeNPs) indeed hold immense potential in various biomedical applications owing to their unique properties. Their biocompatibility, bioavailability, and low toxicity make them particularly promising for use in medical treatments and products. One notable advantage of SeNPs is their compatibility with human organs and tissues, especially when they are synthesized using biological methods. This means that they are less likely to cause adverse reactions or harm to the body, making them safer for use in medical contexts. The size, shape, and synthesis method of SeNPs play crucial roles in determining their effectiveness in biological systems. These factors can influence their interactions with cells and tissues, as well as their ability to target specific diseases or conditions. For example, SeNPs have shown potential in the treatment of various infections, cancer, and diabetes, as well as in acting as chemopreventive agents, anti-inflammatory agents, and antioxidants.

Biologically synthesized selenium nanoparticles (SeNPs) have demonstrated impressive antimicrobial activity, surpassing that of chemically synthesized SeNPs in some studies. This heightened efficacy against certain pathogens underscores the potential of biologically derived SeNPs in combating microbial infections. Moreover, the use of SeNPs in treating chronic and nosocomial infections holds promise due to their low toxicity and compatibility with biological systems. These properties make SeNPs an attractive option for developing alternative strategies against persistent and hospital-acquired infections.

The antimicrobial action of SeNPs is primarily attributed to their ability to inhibit the growth of both bacteria and fungi. This broad-spectrum activity is valuable in addressing antimicrobial resistance, a global health concern that demands innovative solutions beyond conventional antibiotics. By targeting microbial pathogens through unique mechanisms, SeNPs offer a promising avenue for overcoming antimicrobial resistance.

The biosynthesis of selenium nanoparticles (SeNPs) using Azadirachta indica extract represents an innovative approach harnessing the bioactive compounds present in neem for the production of nanomaterials with potential biomedical applications.

Azadirachta indica, commonly referred to as neem, is indeed a remarkable tree belonging to the mahogany family Meliaceae. Native to the Indian subcontinent and parts of Southeast Asia, neem has become naturalized and cultivated in tropical and subtropical regions across the globe. This versatile plant is renowned for its multifaceted properties, including

insecticidal, antibacterial, antifungal, and anti-inflammatory attributes. Neem trees can grow to heights of 15–20 meters, featuring evergreen leaves and fragrant white flowers. Its fruits and seeds serve as the primary sources of neem oil, which boasts a myriad of medicinal and therapeutic applications.

Throughout history, neem has played a pivotal role in traditional Chinese, Ayurvedic, and Unani medicinal systems, revered for its efficacy in treating and preventing various ailments. This is attributed to its rich composition of bioactive compounds such as nimbin, nimbidin, nimbolide, and limonoids.Research has elucidated numerous health benefits associated with neem, ranging from scavenging free radicals to managing cancer and modulating genetic pathways. Its antioxidant properties contribute to the scavenging of free radicals, which are implicated in various diseases and aging processes. Moreover, neem has shown promise in anticancer management, with studies highlighting its potential in inhibiting tumor growth and metastasis.

In this present study, the Azadirachta indica extract was used as reducing and capping agent to synthesize selenium nanoparticles. The synthesized SeNPs was characterized using UV-Visible spectrophotometer and tested for its biomedical application involving antimicrobial activity and zebrafish embryonic toxicology evaluation.

Materials and methods

Preparation of *Azadirachta indica* extract:

In this study, *Azadirachta indica* extract is used as a reducing and capping agent to synthesize selenium nanoparticles. To prepare the extract, 1g of *Azadirachta indica* was measured and added to 100 mL distilled water. Then it was boiled using heating mantle at 60-70 ^{o}C for 20 minutes. The boiled extract was then filtered using Whatmann No:1 filter paper. The filtered extract was kept for selenium nanoparticle synthesis.

Green synthesis of selenium nanoparticles

20mM sodium selenite was measured and added to conical flask containing 60mL distilled water. To that, 40mL filtered *Azadirachta indica* extract was added. The reaction mixture was kept on a magnetic stirrer for about 700 rpm for 48h. The synthesized selenium nanoparticle solution was centrifuged at 8000 rpm for 10 minutes to separate pellet out of supernatant. The collected pellet was stored in an airtight Eppendorff tube for further characterization and biomedical activities.

Characterization:

The green synthesized selenium nanoparticles was characterized using Double beam-UV-Visible spectrophotometer

Antimicrobial activity

The antimicrobial activity of the green synthesized selenium nanoparticles was evaluated using the agar well diffusion technique. Mueller Hinton agar plates were prepared and sterilized using an autoclave at 121ºC for 15- 20 minutes. After sterilization, the medium was poured on to the surface of sterile Petri plates and allowed to cool to room temperature. The bacterial suspension (*Streptococcus mutans, Escherichia coli, Staphyloccus aureus, Candida albicans*) was spread evenly onto the agar plates using sterile cotton swabs. Wells of 9mm diameter were created in the agar plates using a sterile polystyrene tip. The wells were then filled with different concentrations (25 µg, 50 µg, 100 µg) of SeNPs . An antibiotic (e.g., Bacteria-Amoxyrite, Fungi- Flucanazole) was used as a standard. The plates were incubated at 37°C for 24 hours and 48 hours for fungal cultures. The antimicrobial activity was evaluated by measuring the diameter of the inhibition zone surrounding the wells. The diameter of the zone of inhibition was measured using a ruler and recorded in millimeters (mm) and the zone of inhibition was calculated.

Evaluation of acute cytotoxicity using zebrafish embryos

Wild-type zebrafish were obtained from local vendors in India and kept in separate tanks with controlled conditions of temperature, light/dark cycle, and pH. They were fed dry blood worms or optimal food twice daily. Zebrafish embryos were collected by breeding one female with three males in a tank, and viable eggs were rinsed with E3 medium. The embryos were placed in culture plates with different well sizes and treated with varying concentrations of green synthesized selenium nanoparticles. The experiment was replicated three times, with control groups included. Dead embryos from the treated groups were removed every 12 hours. The plates were covered with foil and kept at 28°C.

Zebrafish embryo evaluation :

During the observation period after fertilization, the growth of Zebrafish embryos was monitored with a stereo microscope. The embryos were exposed to different concentrations of green synthesized SeNPs (5, 10, 20, 40, 80 µg/L) for 24-78 hours. The embryo mortality and hatching rates were checked every 24 hours. The study focused on tracking embryo/hatchling mortality, hatching rate, and any abnormalities in both control and treatment groups. Images of deformed embryos were taken using a COSLAB -Model: HL-10A light microscope, and the percentage of abnormal embryos was documented daily.

Results:

UV-Visible spectroscopy:

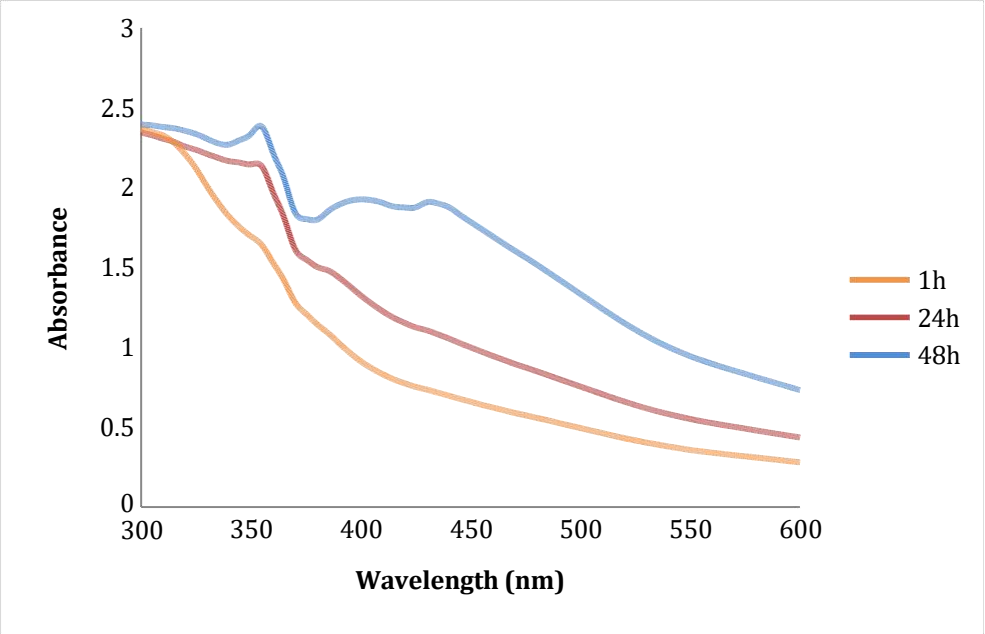

Figure 1: UV-Visible spectra of *Azadirachta indica* mediated selenium nanoparticles

The UV-Visible spectra of Azadirachta indica (A. indica) mediated selenium nanoparticles (SeNPs) revealed a distinct surface plasmon resonance (SPR) peak at 360 nm, indicative of the nanoparticles optical properties. The presence of the SPR peak at 360 nm suggests the excitation of surface plasmons within the structure of the selenium nanoparticles. The specific wavelength of the SPR peak is influenced by various factors, such as nanoparticle size, shape, and composition. In the case of SeNPs synthesized with A. indica, the presence of organic compounds or biomolecules from the plant extract likely contributes to the formation and stabilization of the nanoparticles, thereby affecting their optical properties.

Antimicrobial activity:

Figure 2: Evaluation of antimicrobial activity of green synthesized SeNPs using agar well diffusion technique

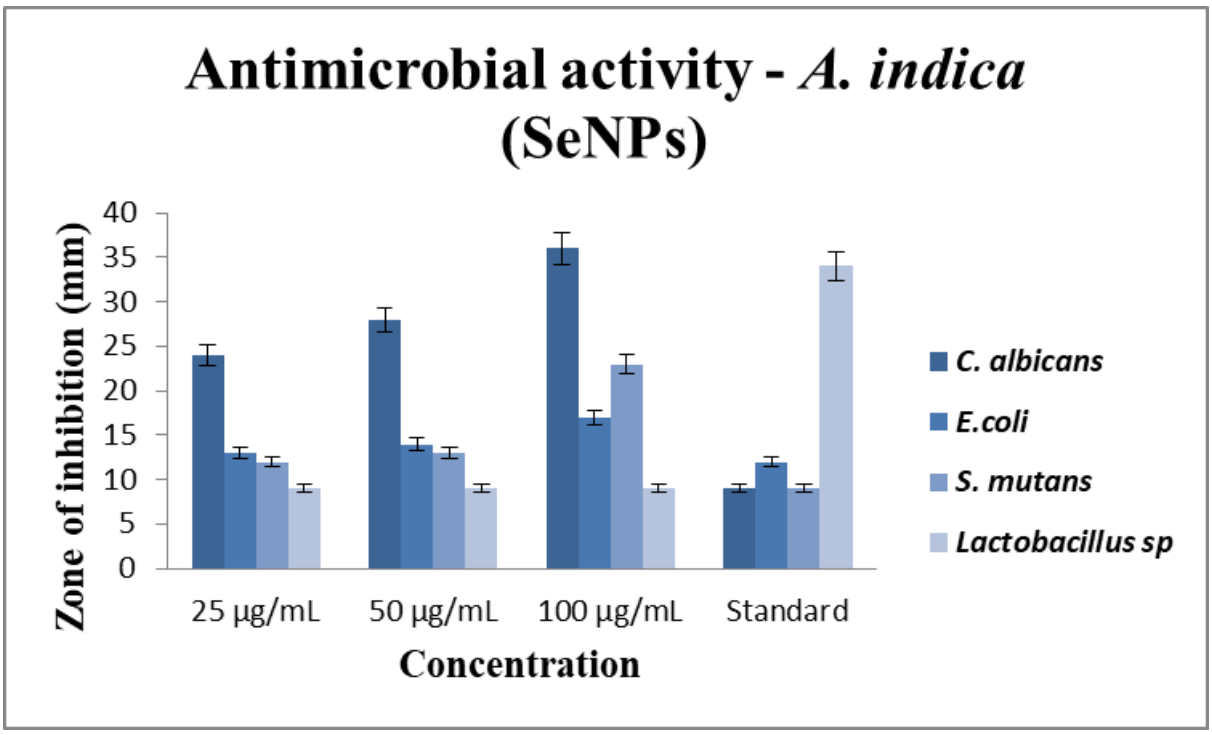

Figure 3: Antimicrobial activity of green synthesized SeNPs against different oral pathogens

The antimicrobial activity of A. indica mediated selenium nanoparticles (SeNPs) against oral pathogens at different concentrations (25 µg/mL, 50 µg/mL, and 100 µg/mL) was tested and also compared to a standard (amoxyrite). The oral pathogens tested include C. albicans, E. coli, S. mutans, and Lactobacillus sp. The results show that at all concentrations, the SeNPs exhibited varying degrees of antimicrobial activity against the tested pathogens.

For C. albicans, the highest inhibition was observed at 100 µg/mL with a zone of inhibition of 36 mm, followed by 50 µg/mL (28 mm) and 25 µg/mL (24 mm), compared to the standard with a zone of inhibition of 9 mm. Similarly, for E. coli, the SeNPs showed inhibition zones of 17 mm, 14 mm, and 13 mm at 100 µg/mL, 50 µg/mL, and 25 µg/mL, respectively, compared to the standard with a zone of inhibition of 12 mm.

In the case of S. mutans, the highest inhibition was observed at 100 µg/mL with a zone of inhibition of 23 mm, followed by 50 µg/mL (13 mm) and 25 µg/mL (12 mm), compared to the standard with a zone of inhibition of 9 mm. Lastly, for Lactobacillus sp, the SeNPs showed consistent inhibition zones of 9 mm at all concentrations, while the standard exhibited a larger zone of inhibition of 34 mm.

Overall, the results suggest that A. indica mediated SeNPs possess significant antimicrobial activity against the tested oral pathogens, with varying levels of effectiveness at different concentrations. These findings highlight the potential of SeNPs as a promising antimicrobial agent for combating oral infections caused by these pathogens.

Embryonic toxicology:

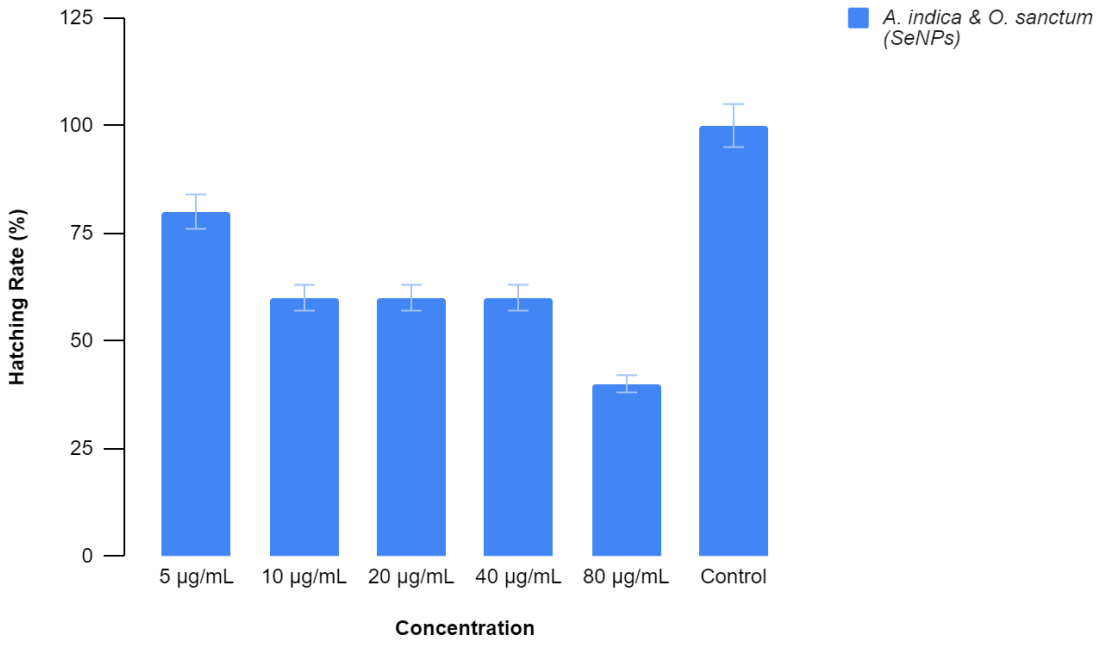

Figure 4: Hatching rate of zebrafish embryos treated with SeNPs

In the study of embryonic toxicology involving A. indica mediated selenium nanoparticles (SeNPs), the hatching rates of the embryos were evaluated at different concentrations of SeNPs. The results showed that at a concentration of 5 µg/mL, the hatching rate was 80%, indicating a relatively high hatching success. However, as the concentration of SeNPs increased to 10 µg/mL, 20 µg/mL, 40 µg/mL, the hatching rates decreased to 60% for each concentration, suggesting a dose-dependent decrease in hatching success. Notably, at the highest concentration of 80 µg/mL, the hatching rate dropped to 40%, indicating a significant impact on the embryos' viability. In comparison, the control group, which did not receive SeNPs treatment, exhibited a hatching rate of 100%, indicating normal embryonic development in the absence of SeNPs. These findings suggest that exposure to increasing concentrations of SeNPs negatively affects the hatching success rate of zebrafish embryos, highlighting the potential embryotoxic effects of SeNPs.

Viability rate:

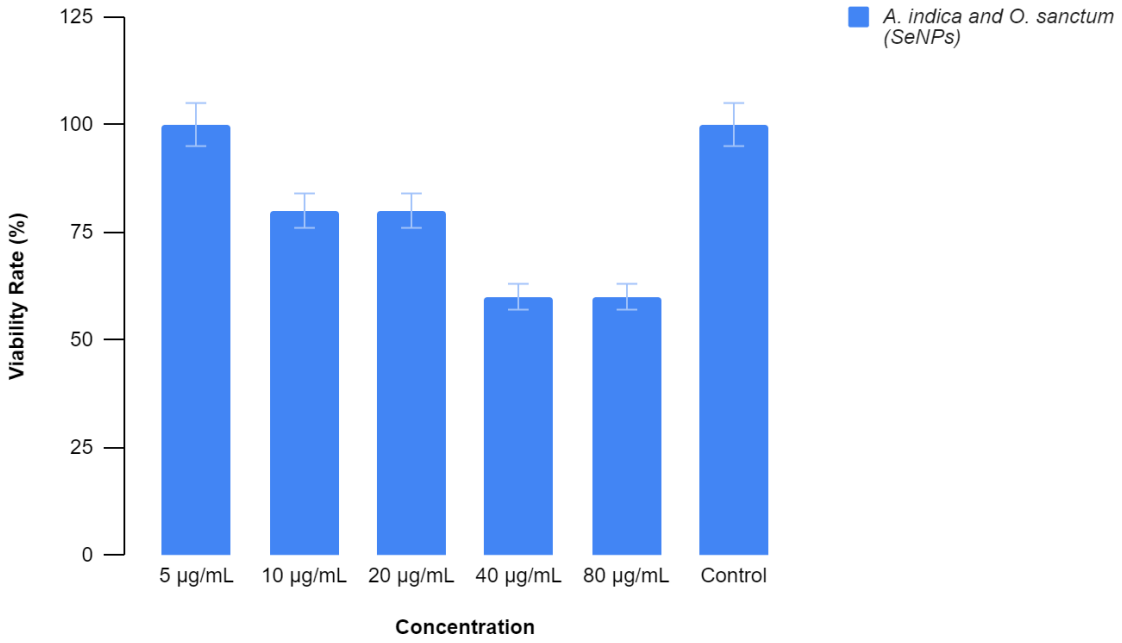

Figure 5: Viability rate of zebrafish embryos treated with SeNPs

In the embryonic toxicology study involving A. indica mediated selenium nanoparticles (SeNPs), the viability rates of the embryos were assessed at different concentrations of SeNPs. The viability rates were compared to a control group that was not exposed to SeNPs. At a concentration of 5 µg/mL, the viability rate was observed to be 100%, indicating that the embryos were fully viable at this concentration. However, as the concentration of SeNPs increased to 10 µg/mL, the viability rate decreased to 80%, suggesting a slight decrease in viability compared to the lower concentration. This trend continued as the concentration of SeNPs further increased to 20 µg/mL and 40 µg/mL, with the viability rate remaining at 80% and then dropping to 60%, respectively. At the highest concentration tested, 80 µg/mL, the viability rate remained at 60%, indicating a consistent decrease in viability at higher concentrations of SeNPs.

The control group, which was not exposed to SeNPs, maintained a viability rate of 100%, serving as a baseline for comparison. These results suggest that the viability of embryos treated with SeNPs is concentration-dependent, with higher concentrations leading to a decrease in viability.

Discussion:

The present study investigated the antimicrobial activity and embryotoxic effects of A. indica mediated selenium nanoparticles (SeNPs). The findings reveal significant antimicrobial potential against oral pathogens, alongside notable impacts on embryonic development, shedding light on both the therapeutic and potential risks associated with SeNPs.

The results demonstrated the efficacy of A. indica mediated SeNPs against a range of oral pathogens, including C. albicans, E. coli, S. mutans, and Lactobacillus sp. The observed zone of inhibition indicated a dose-dependent response, with higher concentrations of SeNPs generally correlating with larger inhibition zones. Particularly, the substantial inhibition of C. albicans and E. coli, suggesting SeNPs as a promising agent against common oral infections.

Additionally, the UV-Visible spectra analysis of A. indica mediated SeNPs revealed a distinctive surface plasmon resonance (SPR) peak at 360 nm. This characteristic peak signifies the nanoparticles optical properties, providing valuable insights into their structural characteristics and confirming successful synthesis.

Selenium nanoparticles (SeNPs) have emerged as promising antimicrobial agents against a spectrum of oral pathogens, encompassing Streptococcus mutans, Porphyromonas gingivalis, Staphylococcus aureus, and Lactobacillus species . Although the precise mechanisms underpinning SeNPs antimicrobial action remain incompletely elucidated, existing literature points towards several plausible avenues.

Studies suggest that SeNPs may disrupt bacterial cell membranes, trigger the generation of reactive oxygen species (ROS), and interfere with pivotal bacterial enzyme functions . Notably, investigations into the inhibitory effect of SeNPs on P. gingivalis underscored a concentration-dependent relationship, intimating that higher SeNP concentrations could potentiate bactericidal effects. However, even at the highest tested concentration, complete eradication of the bacteria was not achieved, hinting at the involvement of alternative antimicrobial mechanisms. SeNPs have been observed to impede bacterial enzyme functions involved in the synthesis of extracellular polysaccharides (EPS), integral components of biofilms crucial for bacterial adhesion and virulence . By disrupting EPS production, SeNPs may hinder biofilm formation, thereby mitigating bacterial colonization and the progression of oral infections.

Moreover, SeNPs have been shown to induce the generation of ROS, which can inflict damage upon bacterial cell membranes and DNA, ultimately culminating in bacterial demise.

ROS-mediated disruption may extend to bacterial enzyme activities, thereby augmenting SeNPs antimicrobial efficacy[-21].

The zebrafish embryotoxicity assessment revealed concentration-dependent impacts on zebrafish embryo hatching rates and viability. While lower concentrations of SeNPs (5 µg/mL) did not significantly affect hatching or viability, higher concentrations exhibited a clear dose-dependent decrease in both parameters. The observed decrease in hatching rates and viability suggests a potential adverse effect on embryonic development with increasing SeNP concentrations. These findings raise concerns regarding the safety of SeNPs, particularly at higher doses, and emphasize the importance of thorough toxicity evaluations in nanomedicine research.

Selenium nanoparticles (SeNPs) have emerged as a promising avenue for various health benefits, owing to their reduced toxicity, enhanced bioavailability, and physiological advantages over traditional forms of selenium. Selenium nanoparticles possess high antioxidant, antimicrobial, antidiabetic, and anticancer properties further extend their utility in biomedical and pharmaceutical domains[, 22-26].

Beyond these established benefits, SeNPs have demonstrated potential antiviral effects. Recent research suggests that SeNPs may deactivate viral particles when attached to surfaces, similar to selenium's role in protecting against viral infections. The distinct physical and chemical properties of nanoparticles, including their shape and size, make them attractive for developing novel surface coatings and antiviral substances that could mitigate the transmission of active viral particles[, 27-32].

Overall, this study underscores the multifaceted nature of A. indica mediated SeNPs, highlighting their promising antimicrobial properties against oral pathogens alongside potential embryotoxic effects. These findings contribute to the growing body of knowledge on nanomedicine safety and efficacy, informing future research directions aimed at harnessing the therapeutic potential of SeNPs.

Implications and Future Directions

The dual nature of SeNPs, demonstrating potent antimicrobial activity against oral pathogens but also exhibiting embryotoxic effects, presents a complex scenario for their potential biomedical applications. Further studies are needed to elucidate the underlying mechanisms of SeNPs antimicrobial activity and embryotoxicity.

Conclusion

The study underscores the dual nature of A. indica mediated SeNPs: as a potent antimicrobial agent against oral pathogens and a potential embryotoxic substance. While SeNPs exhibited significant antimicrobial activity against C. albicans and E. coli, surpassing conventional treatments, their impact on zebrafish embryo hatching and viability rates revealed dose-dependent embryotoxic effects. Lower SeNP concentrations demonstrated relatively high embryo viability, but higher concentrations led to decreased hatching and viability rates. The control group maintained normal development, indicating the less adverse effects of increasing SeNP concentrations on embryo viability. This dichotomy emphasizes the necessity for further research to optimize SeNPs therapeutic efficacy against oral infections while mitigating potential risks to embryonic development. Balancing these dual roles will be crucial in harnessing the full therapeutic potential of SeNPs while ensuring their safety for clinical use.

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