Evaluation of Zebrafish Embryonic Toxicology using Mimosa Pudica -Assisted Selenium Nanoparticles

DhavePrasath V M¹, Prabhakaran A K², Rajeshkumar Shanmugam¹*, Dhanyaa Muthukumaran¹

¹Nanobiomedicine Lab, Centre for Global Health Research, Saveetha Medical College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai, India

²Department of Orthopaedics, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, India

Email: rajeshkumars.smc@saveetha.com

The exploration of zebrafish embryonic toxicity using Mimosa pudica (M. pudica)-mediated Selenium Nanoparticles (Se NPs) is a significant area of research that looks at the potential effects of Se NPs on zebrafish embryos. To determine the impact of Se NPs on zebrafish embryonic hatching and viability, this study employed the green synthesis of Se NPs using M. pudica. Se NPs were synthesized using M. pudica extract in the study, and the effects on zebrafish embryos were then examined. The results demonstrated that Se NPs had a considerable impact on zebrafish embryo viability and hatching. The Se NPs significantly outperformed the control group in terms of viability and hatching, indicating that they have the potential to enhance embryonic development and survival. The study also showed that Se NPs had a low level of toxicity, indicating that they were safe to use. This discovery emphasizes the promise of Se NPs supported by M. pudica as a secure medication candidate for a range of applications. The toxicity of zebrafish embryos utilizing M. pudica-mediated Se NPs offers encouraging proof of the beneficial effects of Se NPs on zebrafish embryo viability and hatching. The low levels of toxicity found in this investigation provide additional evidence for the possible safety of Se NPs aided by M. pudica for use in medicinal applications.

Keywords: Embryonic toxicology, Green Synthesis, Selenium Nanoparticles, Eco-friendly method.

1. Introduction

The embryonic development of the zebrafish (Danio rerio) is examined using the embryos of zebrafish as a model organismZebrafish embryos differ from more conventional animal models like rat and mouse embryos in their tiny size, fertilization through the environment, and quick development. They are a useful and economical model for testing for toxins because of these benefits. (Bambino and Chu et.al, 2017). The synthesisedM.pudica-mediated selenium

nanoparticle's toxicity was analyzed via zebrafish embryonic toxicology. The zebrafish models are highly useful in discovering new drugs and their adverse reactions at low maintenance and low cost. (Cassar et al., 2020). M. pudica, commonly called the sensitive plant, has intriguing medical qualities. Different civilizations have long utilized this plant to treat several illnesses, such as diarrhoea, skin infections, and wounds. It evaluated the woundhealing properties of the root of M.pudica. (Kokane et al., 2009).

The potential applications of selenium nanoparticles (SeNPs) in the medicinal field are in cancer treatment and inflammatory and oxidative stress-mediated conditions. (Khurana et al. 2019). Also, there is wide usage in agricultural fields in which it helps in promoting seed germination and adding nutritional valueto crops(Song et al. 2023). However, it is crucial to realize the potential toxicological implications of SeNPs, as with any newly developed technology.

SeNPs' toxicity has been examined in several studies. In one Study, SeNPs' cytotoxicity was assessed in human liver cells. The outcomes demonstrated that cells underwent oxidative stress and lost viability when exposed to high concentrations of SeNPs. (Cui et al. 2018). In the other study done in 2022, when Zebrafish were exposed to selenium nanoparticles the toxicity was higher in the liver than in the gills of the zebrafish(Fan et al. 2022)

An additional 2020 study examined the impact of SeNPs on the male rat reproductive system. The researchers discovered that exposure to SeNPs decreased sperm viability, motility, and count while also increasing DNA damage in the testes (Hamza and Diab 2020). Overall, the physicochemical characteristics of SeNPs, such as size, surface charge, and concentration, determine their toxicological consequences. To comprehend the potential toxicity of SeNPs in many biological systems and to develop mitigation methods for their harmful effects, more research is required. The study aims toevaluate the embryonic toxicology of Mimosa pudicamediated selenium nanoparticles and analyze the toxicity of the Mimosa pudica-mediated selenium nanoparticles.

2. Materials and Methods

2.1 PreparationofM. pudica-mediatedSe NPs

Weigh 1g of M. pudicapowder was mixed with 100ml of distilled water boiled by using a heating mantle and filtered. Sodium selenite was dissolved in 50ml of distilled water to ensure thorough mixing. Then, 50ml of the prepared M. pudica solution was combined with 50ml of the sodium selenite solution.

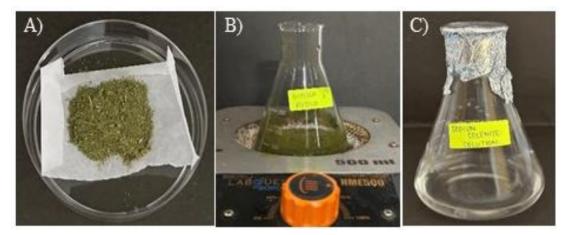


Figure 1: A) M.pudica powder B) M.pudica boiled using heating mantle C) sodium selenite solution

2.2 Zebrafish embryonic toxicology evaluation of selenium nanoparticles

2.2.1Fish maintenance and SeNPs exposure

Wild-type zebrafish (Danio rerio) were acquired from local Indian vendors and were contained in individual tanks under controlled conditions of temperature ($28\pm2C$), light/dark cycle (14:10 h), and pH (6.8-8.5). The fish were fed with commercially available optimum food twice a day. 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. The study involved the placement of fertilized eggs in culture plates of 6 well plates. 10 embryos per 2 mL solution per well. To prepare the experimental treatment, M. pudica-Se NPs with five different concentrations were freshly made and added directly to the E3 medium. Healthy fertilized embryos were exposed to different concentrations of Se NPs ranging from 5 to $80\mu g/mL$ for 24 to 96 hours post-fertilization. Control groups were also included in the experiment. Dead embryos were removed from the nanoparticles exposed groups every 12 hours. All experimental plates were maintained at $28^{\circ}C$.

2.2.2 Zebrafish embryo evaluation

Throughout the exposure period following fertilization, the developmental stages of Zebrafish embryos were monitored using a compound microscope. The embryos were subjected to various concentrations of selenium nanoparticles (5, 10, 20, 40, 80 μ g/mL) for 24-78 hours. 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.

3. Result

3.1 Visual observation



Figure 2: The Reddish orange colour confirmed Se NPs were synthesized using M.pudica

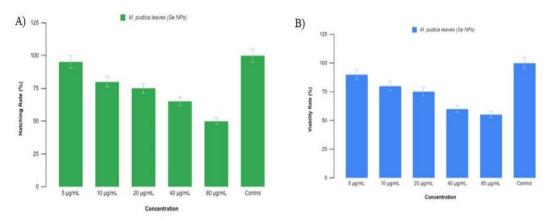


Figure 3: Graphical representation of zebrafish embryos A) Toxicology viability rate
B)Toxicology Hatching rate

The impact of selenium nanoparticles (Se NPs) derived from M. pudica on both the (A) viability rate and (B) hatching rate of zebrafish eggs at varying concentrations. For the viability rate of zebrafish eggs decreases with higher concentrations of Se NPs. The viability is about 95% at 5 μ g/mL, decreases to roughly 70% at 20 μ g/mL, and further reduces to about 50% at 80 μ g/mL, with the control group maintaining a viability rate close to 100%. Similarly the hatching rate, there is a clear dose-dependent decrease observed with increasing concentrations of Se NPs. The hatching rate starts at approximately 95% for 5 μ g/mL and declines progressively to around 50% at 80 μ g/mL, while the control group shows a near 100% hatching rate. These results indicate that higher concentrations of Se NPs have a toxic effect on both the hatching and viability rates of zebrafish eggs.

These findings suggest that selenium nanoparticles have the potential to enhance the development and survival of zebrafish embryos.

4. Discussion

Selenium nanoparticles are having unique characterstics with many biomedical applications ((Jayapriya et al., 2022, Johnson et al., 2024, Shanmugam et al., 2023, Kathiravana et al., 2023). Zebrafish a very useful vertebrae for analyzing the growth and developmental effects of certain synthetic and natural compounds by using zebrafish embryonic toxicology. (Hsieh et al. 2023). Also, Mimosa pudica is toxic in higher doses in chicks orally as mentioned in the study of 2016. (Nghonjuyi et al., 2016). The evaluation of zebrafish embryonic toxicology using M.pudica-assisted selenium nanoparticles has demonstrated promising results. The study assessed the effect of selenium nanoparticles on zebrafish embryonic hatching and viability.

The results showed that selenium nanoparticles had a significant effect on zebrafish embryonic hatching and viability. These findings suggest that selenium nanoparticles have the potential to enhance the development and survival of zebrafish embryos. Already a study conducted in 2018 has identified its mechanism of action as a chemotherapeutic agent(Menon et al. 2018). Even Though there are many therapeutic uses such as antioxidant activity antimicrobial activity anti-diabetic activity anti-inflammatory activity their specific mechanism of action is unknown. (Khurana et al. 2019).

Furthermore, the study demonstrated that selenium nanoparticles had a low toxicity level, indicating their potential as safe substances for use. The observed low toxicity levels support the idea that Mimosa pudica-assisted selenium nanoparticles may serve as a safe therapeutic option.

5. Conclusion

In conclusion, the evaluation of zebrafish embryonic toxicology using M. pudica-mediated selenium nanoparticles provides promising evidence that selenium nanoparticles can improve zebrafish embryonic hatching and viability. This study also highlights the low toxicity levels of Mimosa pudica-mediated selenium nanoparticles favours and further suggests their potential as safe drugs.

References

- 1. Bambino, K., & Chu, J. (2017). Zebrafish in toxicology and environmental health. Current topics in developmental biology, 124, 331-367.https://doi.org/10.1016/bs.ctdb.2016.10.007
- 2. Cassar, S., Adatto, I., Freeman, J. L., Gamse, J. T., Iturria, I., Lawrence, C., ... & Zon, L. I. (2019). Use of zebrafish in drug discovery toxicology. Chemical research in toxicology, 33(1), 95-118. DOI: 10.1021/acs.chemrestox.9b00335
- 3. Cui, D., Liang, T., Sun, L., Meng, L., Yang, C., Wang, L., ... & Li, Q. (2018). Green synthesis of selenium nanoparticles with extract of hawthorn fruit induced HepG2 cells apoptosis. Pharmaceutical biology, 56(1), 528-534. https://doi.org/10.1080/13880209.2018.1510974
- 4. Fan, S., Yang, Y., Sun, L., Yu, B., Dai, C., & Qu, Y. (2022). Different toxicity to liver and gill of zebrafish by selenium nanoparticles derived from bio/chemical methods. Environmental Science and Pollution Research, 29(40), 61512-61521.
- 5. Hamza, R. Z., & Diab, A. E. A. A. (2020). Testicular protective and antioxidant effects of

- selenium nanoparticles on Monosodium glutamate-induced testicular structure alterations in male mice. Toxicology Reports, 7, 254-260. https://doi.org/10.1016/j.toxrep.2020.01.012
- 6. Hsieh, J. H., Nolte, S., Hamm, J. T., Wang, Z., Roberts, G. K., Schmitt, C. P., & Ryan, K. R. (2023). Systematic Evaluation of the Application of Zebrafish in Toxicology (SEAZIT): developing a data analysis pipeline for the assessment of developmental toxicity with an interlaboratory study. Toxics, 11(5), 407. https://doi.org/10.3390/toxics11050407
- 7. Khurana, A., Tekula, S., Saifi, M. A., Venkatesh, P., & Godugu, C. (2019). Therapeutic applications of selenium nanoparticles. Biomedicine & Pharmacotherapy, 111, 802-812. https://doi.org/10.1016/j.biopha.2018.12.146
- 8. Kokane, D. D., More, R. Y., Kale, M. B., Nehete, M. N., Mehendale, P. C., & Gadgoli, C. H. (2009). Evaluation of wound healing activity of root of Mimosa pudica. Journal of ethnopharmacology, 124(2), 311-315. https://doi.org/10.1016/j.jep.2009.04.038
- 9. Adewuyi, Reuben, et al. "Prediction of Impact Strength of TIG Welded Cr-Mo Steel Using Artificial Neural Networks." Metallurgical and Materials Engineering 30.1 (2024): 61-69.
- 10. Barhoum, A., & Makhlouf, A. S. H. (2018). Emerging Applications of Nanoparticles and Architecture Nanostructures (pp. 255-278). Amsterdam, The Netherlands:: Elsevier.
- 11. Menon, S., Ks, S. D., Santhiya, R., Rajeshkumar, S., & Kumar, V. (2018). Selenium nanoparticles: A potent chemotherapeutic agent and an elucidation of its mechanism. Colloids and Surfaces B: Biointerfaces, 170, 280-292.https://doi.org/10.1016/j.colsurfb.2018.06.006
- 12. Nghonjuyi, N. W., Tiambo, C. K., Taïwe, G. S., Toukala, J. P., Lisita, F., Juliano, R. S., & Kimbi, H. K. (2016). Acute and sub-chronic toxicity studies of three plants used in Cameroonian ethnoveterinary medicine: Aloe vera (L.) Burm. f.(Xanthorrhoeaceae) leaves, Carica papaya L.(Caricaceae) seeds or leaves, and Mimosa pudica L.(Fabaceae) leaves in Kabir chicks. Journal of ethnopharmacology, 178, 40-49.https://doi.org/10.1016/j.jep.2015.11.049
- Song, J., Yu, S., Yang, R., Xiao, J., & Liu, J. (2023). Opportunities for the use of selenium nanoparticles in agriculture. NanoImpact, 31, 100478. https://doi.org/10.1016/j.impact.2023.100478
- 14. Shridhar, N. B., Nārāyaṇa, K., & Yathiraj, S. (2015). Toxicity studies of Mimosa pudica in livestock and laboratory animals. LAP Lambert Academic Publishing.
- 15. Wang, H., Wang, Y., Wang, Q., Lv, M., Zhao, X., Ji, Y., ... & Chen, L. (2022). The combined toxic effects of polyvinyl chloride microplastics and di (2-ethylhexyl) phthalate on the juvenile zebrafish (Danio rerio). Journal of Hazardous Materials, 440, 129711. https://doi.org/10.1016/j.jhazmat.2022.129711
- Zambonino, M. C., Quizhpe, E. M., Jaramillo, F. E., Rahman, A., Santiago Vispo, N., Jeffryes,
 C., & Dahoumane, S. A. (2021). Green synthesis of selenium and tellurium nanoparticles:
 current trends, biological properties and biomedical applications. International Journal of Molecular Sciences, 22(3), 989. https://doi.org/10.3390/ijms22030989.
- 17. Qu, Xiaoqing, et al. "Effect of oat β-glucan on in vitro digestion characteristics of set-type yogurt." Acta Innovations (2022).
- 18. Kathiravan, A., Udayan, E., Rajeshkumar, S. et al. Unveiling the Biological Potential of Mycosynthesized Selenium Nanoparticles from Endophytic Fungus Curvularia sp. LCJ413. BioNanoSci. (2023). https://doi.org/10.1007/s12668-023-01223-w.
- 19. Shanmugam R, Anandan J, Balasubramanian A K, et al. (December 20, 2023) Green Synthesis of Selenium, Zinc Oxide, and Strontium Nanoparticles and Their Antioxidant Activity A Comparative In Vitro Study. Cureus 15(12): e50861. doi:10.7759/cureus.50861.
- 20. Dorkyor, Thitipoom, et al. "A Development of Gold Nanodots Fabrication for Optical Plasmonic Applications." Nanotechnology Perceptions 20.2 (2024): 1-24.