International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2024: 7.101

Green-Synthesized Zinc Oxide Nanoparticles Enhance Tail Fin Regeneration in *Gambusia affinis*

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Running title: *Effect of green synthesized zinc oxide nanoparticles on fish tail fin regeneration*

Abstract: ***The present study explores the regenerative effects of green-synthesized zinc oxide nanoparticles (ZnO NPs), derived from pomegranate leaf extract, on tail fin regeneration in Gambusia affinis. Fish were subjected to tail fin amputation and exposed to varying concentrations (10, 20, and 30 µg/mL) of ZnO NPs over a 96-hour period. At lower concentrations, ZnO NPs enhanced regeneration, with 10 µg/mL showing the most sustained effect. However, higher doses initially accelerated regrowth but later hindered the process, likely due to cytotoxic effects. The findings underscore the biomedical potential of plant-mediated ZnO NPs in tissue regeneration, while also emphasizing the importance of optimal dosing.***

Keywords: green synthesis, *Gambusia affinis,* nanotoxicity, tail fin regeneration, zinc oxide nanoparticles

1. Introduction

Green synthesis of nanoparticles has emerged as a promising alternative to conventional physical and chemical methods, due to its simplicity, sustainability, and eco-friendliness [1,2]. Among various biological sources, plant-mediated synthesis using leaf extracts has gained popularity owing to the rich presence of phytochemicals such as flavonoids, tannins, polyphenols and alkaloids, which act as natural reducing and stabilizing agents during nanoparticle formation [3,4]. Pomegranate (*Punica granatum*) leaves extract has been widely recognized for its high content of bioactive compounds, including polyphenols, ellagic acid, and flavonoids. These phytochemicals facilitate the reduction of metal ions to nanoparticles with enhanced stability and biological properties [5,6]. Zinc oxide nanoparticles (ZnO NPs) synthesized using pomegranate leaf extracts exhibit excellent biocompatibility, antioxidant activity, and antibacterial potential [7]. Besides these activities, since these NPs are less or nontoxic as generated from edible plant material that has natural healing, antioxidant, anti-inflammatory, tissue formation, and cell growth bio- potential [1, 2].

Regeneration is a biological phenomenon where organisms have the ability to restore or replace damaged, lost, or amputated tissues, organs, or body parts. This process involves the reactivation of cellular mechanisms that promote tissue growth, ensuring the recovery of normal structure and function. The capability for regeneration varies across species; some organisms like certain amphibians and fish possess a remarkable ability to regenerate entire limbs or fins, while others may regenerate only specific tissues. Regeneration is not only crucial for survival after injury but also serves as an adaptive advantage, enhancing the organism's ability to maintain homeostasis and continue normal physiological activities [8]. Regeneration studies have become increasingly significant due to its potential applications in regenerative medicine, tissue engineering, and therapeutic approaches aimed at repairing human tissues or treating degenerative diseases. Studying organisms with strong regenerative abilities provides valuable insights into cellular behavior, gene expression, and molecular pathways involved in tissue renewal [9,10].

Gambusia affnis (mosquito fish) is a small fish native to the fresh water. Adult *Gambusia* are generally observed in shallow open waters while immature fish tend to aggregate in shallow vegetated areas. These *Gambusia* exhibit remarkable epimorphic regeneration capacity and has been extensively explored for regeneration studies due to its easy availability, low maintenance, easy manipulation and observation. In aquatic organisms, particularly *Gambusia affinis*, tail fin regeneration is a vital indicator of physiological recovery and tissue repair mechanisms [11,12].

The present study investigates the role of green-synthesized ZnO nanoparticles using pomegranate leaf extract in the regeneration of amputated tail fins in *Gambusia affinis*. The research aims to evaluate the concentration-dependent effects of these nanoparticles, assessing both their potential regenerative benefits and toxicological impacts over initial 96 hours post amputation. This study provides foundational evidence on the potential of plant-mediated nanoparticles in

regenerative biology and contributes to the growing field of eco-nanomedicine.

2. Material and Methods

Collection and tail fin amputation of *Gumbusia affinis*

Gambusia affinis were collected with the help of a fishing net from the lotus tank of the Sangamner Nagarpalika Arts, D. J. Malpani Commerce and B. N. Sarda Science College (Autonomous), Sangamner, Maharashtra. After getting the fish to the laboratory, same size fish were selected for the further study. Fish were acclimatized in glass containers containing dechlorinated water for 24 hours (25 ± 2°C and pH 7.0–7.5) before the tail fin amputation. Fish were fed twice daily with zooplankton, and water was renewed regularly to maintain optimum conditions. Tail fin amputation was carried out with precision to ensure consistency across all experimental fish specimens. A slide with single fish at a time, was placed on a graph paper for reference to accurately measure the amputation point. Using a sterilized sharp surgical blade, the tail fin was amputated, leaving exactly 2 mm of fin tissue from the caudal peduncle [13]. A magnifying glass was utilized during the process to enhance visibility and ensure precision in cutting, avoiding unnecessary damage to surrounding tissues. After amputation, the fish were gently transferred to recovery tanks containing clean dechlorinated water and further exposed to the varied experimental treatments.

Preparation of various treatment doses of ZnO NPs

Characterized and confirmed ZnO NPs [7], fabricated using pomegranate leaves, were obtained from the Zoology Department of the college, carefully transferred into sterile labeled containers and transported under controlled conditions to ensure purity and stability of the same. Proper storage was maintained until further experimental application. A homogenous stock solution of ZnO NPs (1 mg/mL) was prepared in autoclaved distilled water. The NPs were uniformly dispersed in the solution using a stirrer and used for preparing varied doses of ZnO NPs such as 10 µg/mL, 20 µg/mL and 30 µg/mL, for treatment of fish with regenerating tail fins.

The total volume of the solution in each beaker with or without NPs was 25 mL with 10 fish. The beaker with tail fin amputated fish without any ZnO NPs served as controls. The process of regeneration was monitored and measured after every 24 hour, 48 hour, 72 hour and 96 hour. The treatment and regeneration was followed for 8 days post-amputation. Also, the rate of mortality in any beaker (control or treated) was recorded. Statistical analysis (one way ANOVA) of the observations was carried out to compare regeneration rates across different treatment groups. All the experiments were carried out at least thrice.

3. Results

The study evaluated the regenerative potential of green-synthesized ZnO NPs on the regeneration of amputated tail fin of *Gambusia affinis* over a period of time in the comparison with the respective controls. Control group (without ZnO NPs) exhibited natural regeneration with an average fin regrown length of 1.8 ± 0.2 cm by the end of the 8-day post-amputation period. The fish group treated with 10 µg/mL ZnO-NPs showed enhanced regeneration rate as compared to the controls, with a significant increase in fin regrowth measuring 2.8 ± 0.12 cm. Fish treated with either 20 µg/mL or 30 µg/mL ZnO NPs exhibited an average regrowth of 2.3 ± 0.15 cm and 1.9 ± 0.18 cm, respectively.

24 hour time point: During the observation period of 24 hours, it was noted that in the control group, some fish exhibited remarkable tail fin regeneration, with the measured lengths within range of 0.2 cm to 1 cm (average 0.42 ± 0.17 cm). At the same time, a few fish in the control group also showed no noticeable regeneration within this time frame.

The fish exposed to 10 µg/mL ZnO NPs also demonstrated regeneration rates (average 0.38 ± 0.11 cm) comparable to the controls (0.42 ± 0.17 cm), whereas fish treated with 20 µg/mL ZnO NPs exhibited tail fin regeneration with an average of 0.32 ± 0.021 cm and at 30 µg/mL ZnO NPs dose, average 0.34 ± 0.08 cm were recorded (Figure 1).

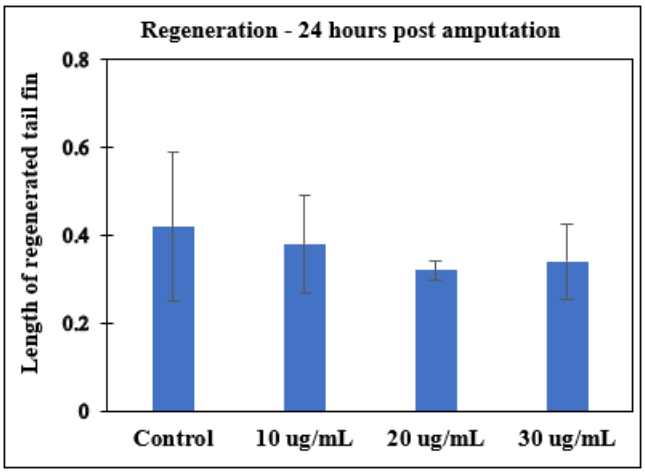

Figure 1: Regeneration of tail fins after 24 hours in either control, 10 µg/mL, 20 µg/mL or 30 µg/mL ZnO NP treated groups: Note more rate of regeneration in control at this stage as compared to other groups, though it was not statistically significant.

48 hour time point: At the end of 48-hours post amputation of tail fins, notable variations in tail fin regeneration as compared to controls were recorded across the experimental groups. The length of the tails fins of control groups, on an average, was extended from 0.42 to 0.56 cm within these 24 hours. This demonstrates a moderate level of natural regenerative capability in *Gambusia affinis* under standard conditions. Fish exposed to either 10 µg/mL, 20 µg/mL or 30 µg/mL of ZnO NPs exhibited variable regeneration patterns as compared to first 24 hours readings (Figure 1), lengths shifting from 0.38 to 0.46 cm, 0.32 to 0.5 cm and 0.34 to 0.76 cm, respectively (Figure 2). In this way, notable regeneration was recorded in the 30 µg/mL ZnO NPs-treated fish, suggesting positive effect of green synthesized ZnO NPs on overall regeneration process at the highest tried dose.

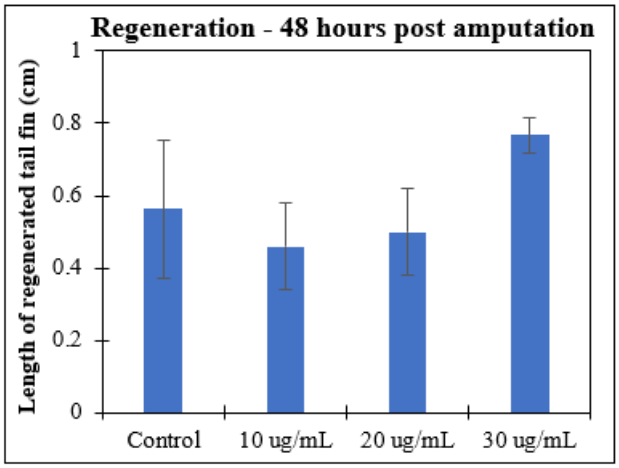

Figure 2: Regeneration of tail fins after 48 hours in either control, 10 µg/mL, 20 µg/mL or 30 µg/mL ZnO NP treated groups: Note *overall enhancement in the lengths of tail fins in all the groups with highest regeneration in 30 µg/mL ZnO NP treated group.*

72 hour time point: By 72-hour regeneration period, the ZnO NPs were found to elevate the regeneration process in all the treated groups as compared to the control. Within 72 hours, the amputated tail fins (0.2 cm) were found to regenerate the lost fins up to 0.61 cm in control group, 0.8 cm.in 10 µg/mL ZnO NPs-, 0.78 cm in 20 µg/mL ZnO NPs- and more than 1 cm in 30 µg/mL ZnO NPs-treated groups. In this way the pattern of highest regeneration at highest tried concentration, observed at 48 hours continued till 72 hours and was statistically significant as compared to other groups (Figure 3). This may be due to increased bioavailability and stimulatory effects of the nanoparticles at elevated concentrations.

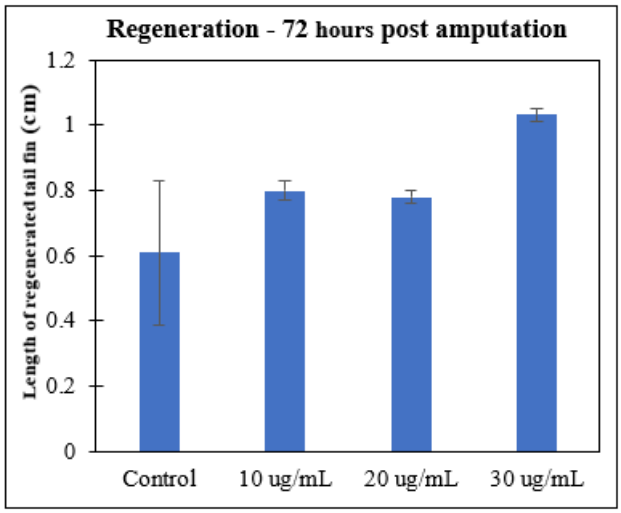

Figure 3: Regeneration of tail fins after 72 hours in either control, 10 µg/mL, 20 µg/mL or 30 µg/mL ZnO NP treated groups: Note overall enhancement in the lengths of tail fins in all the treated groups with highest regeneration in 30 µg/mL ZnO NP treated group.

96 hour time point: At the end of 96-hour observation period, distinct differences in tail fin regeneration patterns were observed among the control and ZnO NP treated groups of *Gambusia affinis*. At this time point the average length of regenerated tail fins in the control group were found to 0.87 (± 0.28) cm which were comparable with the 20 µg/mL ZnO NPs-treated group (0.96 ± 0.02 cm). On the contrary, 10 µg/mL ZnO NP- and 30 µg/mL ZnO NP-treated groups, demonstrated statistically significant enhanced regeneration capacity with 1.45 ± 0.05 cm and 1.4 ± 0.23 cm, respectively.

ZnO NPs may exert a stimulatory effect on cellular proliferation, possibly through the activation of growth factors, enhanced fibroblast activity, or modulation of reactive oxygen species (ROS) at non-toxic levels, thereby promoting tissue repair and regeneration. When the average tail fin length enhancement from 72 to 96 hours for 20 µg/mL (0.78 to 0.96 cm) and 30 µg/mL ZnO NPs (1.03 to 1.4 cm) appeared to be comparable to the control during this period (0.61 to 0.87 cm) whereas 10 µg/mL ZnO NPs seemed to enhance tail fin length gradually in a remarkable manner throughout with a major shift from 0.8 cm to 1.4 cm in these last 24 hours. The lack of regeneration enhancement at higher doses may have gone down with time due to either onset of cytotoxic effects or attributed to a balance between stimulatory and inhibitory mechanisms, where mild toxicity begins to counteract the beneficial proliferative effects observed at lower concentrations.

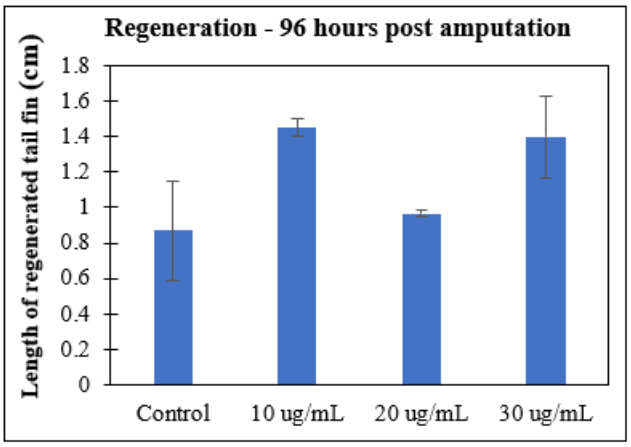

Figure 4: Regeneration of tail fins after 96 hours in either control, 10 µg/mL, 20 µg/mL or 30 µg/mL ZnO NP treated groups: Note overall enhancement in the lengths of tail fins in all the treated groups with highest regeneration in 10 µg/mL ZnO NP treated group. As compared to earlier time points, less enhancement of tail fin lengths were recorded in 20 µg/mL and 30 µg/mL ZnO NP-treated groups.

Mortality check of ZnO NPs: Though the ecofriendly synthesized ZnO NPs were found to accelerate tail fin regeneration process significantly, in order to check the mortality induced in *Gambusa affinis* due to these NPs, live and dead animals were counted at the end of the experiment. About 30% mortality was recorded in both control and 10 µg/mL ZnO NP-treated groups, suggesting 10 µg/mL ZnO NP to be a non-lethal and safe dose for the animals. On the other hand, near about 50% and 80% deaths of *Gambusia affinis* occurred at 20 µg/mL and 30 µg/mL ZnO NP-treated groups, respectively. Thus, the ZnO NPs generated using pomegranate leaf extract appeared to have beneficial and non-lethal effects at lower doses.

4. Discussion

The present study explored the impact of green synthesized, using pomegranate leaf extract, ZnO NPs on the tail fin

regeneration of *Gambusia affinis*. The pomegranate leaf extract acted as both a reducing and stabilizing agent, making the NPs more biocompatible [7]. *Gambusia affinis* is known for its ability to regenerate tail fin, for which it has been explored in many regeneration studies [11,12]. The findings of the present work revealed that at lower concentrations, particularly at 10 µg/mL, the fabricated ZnO NPs significantly enhanced the rate of tail fin regeneration as compared to the control group. These positive effects on the regeneration ability are likely due to the essential role of zinc ions in promoting cell proliferation, collagen synthesis, and tissue repair [14]. Additionally, the presence of bioactive compounds like flavonoids and polyphenols in the pomegranate extract may have contributed to reducing oxidative stress at the wound site, creating a favorable environment for regeneration [6].

The baseline regeneration, observed in the control group, is indicative of the species' intrinsic healing mechanisms without any exogenous stimulation. Exposure to green-synthesized ZnO NPs may have influenced the overall regenerative processes by modulating oxidative stress levels, promoting cellular proliferation, and enhancing antioxidant defenses [15]. However, their effects were found to be highly dose-dependent. Green-synthesized ZnO NPs using pomegranate leaf extract can enhance tail fin regeneration in *Gambusia affinis* through multiple biological mechanisms. These nanoparticles are thought to combine the benefits of zinc ions (Zn^{2+}) and bioactive compounds from pomegranate leaves, contributing to faster and more efficient tissue recovery. ZnO NPs provide Zn^{2+} ions, which are essential for cell division and tissue formation, along with activation of matrix metalloproteinase (MMPs) that help in tissue remodeling and regrowth [16]. The proliferation of fibroblasts and epithelial cells promoting new tissue formation, and simultaneous reduction in oxidative damage, preventing apoptosis in the regenerating tissue, is achieved by ZnO NPs [17]. Pomegranate leaf extract contains flavonoids, tannins, and phenolics, which neutralize reactive oxygen species (ROS). Anti-inflammatory properties from pomegranate bioactive compounds might aid in maintaining balanced immune response, preventing tissue damage [5,6]. ZnO NPs have strong antimicrobial properties, preventing bacterial infections that could slow regeneration [7, 18]. This is crucial in aquatic environments where open wounds are vulnerable to microbial contamination [19]. ZnO NPs are known to stimulate the production of vascular endothelial growth factor (VEGF), leading to increased blood vessel formation [7]. Enhanced blood supply improves oxygen and nutrient transport, accelerating fin regrowth. ZnO NPs may enhance the Wnt/β-catenin signaling pathway, a key regulator of tissue regeneration in fish. Zinc influences gene expression related to fin regeneration, ensuring proper differentiation of new cells [20].

However, at the highest concentration tested (30 µg/mL), the regenerative capacity decreased, accompanied by increased mortality. This indicates a concentration-dependent effect, where optimal doses of ZnO NPs stimulate regeneration, but excessive levels lead to cytotoxicity. High concentrations of ZnO NPs are known to release excess zinc ions and generate reactive oxygen species (ROS), causing oxidative stress, cellular damage, and apoptosis [21]. Similar outcomes have been observed in other aquatic species exposed to elevated nanoparticle concentrations [22]. The green synthesis approach appeared to mitigate some toxicity compared to chemically synthesized nanoparticles, owing to the protective role of plant-derived phytochemicals [1,2,7]. Overall, the study suggests that green synthesized ZnO nanoparticles, when used at appropriate concentrations, can effectively enhance tail fin regeneration in fish. Nevertheless, careful consideration of dosage is crucial, and further research is needed to understand the underlying molecular mechanisms driving this regenerative effect. The higher concentrations of ZnO NPs may release more zinc ions which could induce mild cytotoxicity, affecting cellular activities necessary for proper fin regeneration.

5. Conclusion

The present study demonstrates that green-synthesized zinc oxide (ZnO) nanoparticles, mediated by pomegranate leaf extract, significantly enhance tail fin regeneration in *Gambusia affinis* in a concentration-dependent manner. While the control group exhibited normal fin regeneration within 8 days, exposure to increasing concentrations of ZnO nanoparticles (10 µg/mL, 20 µg/mL, and 30 µg/mL) resulted in accelerated fin regrowth within the same time frame, indicating the promotive effect of ZnO nanoparticles on tissue regeneration. However, higher concentrations also exhibited increased fish mortality, suggesting potential cytotoxicity at elevated levels. These findings highlight the dual role of green-synthesized ZnO nanoparticles in promoting fin regeneration while underscoring the importance of optimizing concentration to minimize adverse effects. To date, very few studies have explored the effects of green-synthesized ZnO nanoparticles on regeneration process, highlighting their potential biomedical potentials.

References

[1] Kirubakaran D, Wahid JBA, Karmegam N *et al.* A Comprehensive Review on the Green Synthesis of Nanoparticles: Advancements in Biomedical and Environmental Applications. *Biomedical Materials & Devices 2025. https://doi.org/10.1007/s44174-025-00295-4*

[2] Singh H, Desimone MF, Pandya S, Jasani S, George N, Adnan M, Aldarhami A, Bazaid AS, & Alderhami SA. Revisiting the Green Synthesis of Nanoparticles: Uncovering Influences of Plant Extracts as Reducing Agents for Enhanced Synthesis Efficiency and Its Biomedical Applications. *International journal of nanomedicine. 2023;18:4727–4750. https://doi.org/10.2147/IJN.S419369*

[3] Villagrán Z, Anaya-Esparza LM, Velázquez-Carriles CA, Silva-Jara JM, Ruvalcaba-Gómez JM, Aurora-Vigo EF, Rodríguez-Lafitte E, Rodríguez-Barajas N, Balderas-León I, & Martínez-Esquivias F. Plant-Based Extracts as Reducing, Capping, and Stabilizing Agents for the Green Synthesis of Inorganic Nanoparticles. *Resources*, *2024; 13*(6):70. https://doi.org/10.3390/resources13060070

[4] Karunakaran G, Sudha KG, Ali S & Cho EB. Biosynthesis of Nanoparticles from Various Biological Sources and Its Biomedical Applications. *Molecules*

(Basel, Switzerland). 2023;28(11):4527 https://doi.org /10.3390/molecules28114527

[5] Yu M, Gouvinhas I, Chen J, Zhu Y, Deng J, Xiang Z, Oliveira P, Xia C, Barros A. Unlocking the therapeutic treasure of pomegranate leaf: A comprehensive review on phytochemical compounds, health benefits, and future prospects, *Food Chemistry: X. 2024; 23:101587.https://doi.org/10.1016/j.fochx.2024.10158 7.*

[6] Saparbekova AA, Kantureyeva GO, Kudasova DE, Konarbayeva ZK, & Latif AS. Potential of phenolic compounds from pomegranate (*Punica granatum L.)* by-product with significant antioxidant and therapeutic effects: A narrative review. *Saudi journal of biological sciences. 2023; 30(2): 103553. https://doi.org/10.1016/j.sjbs.2022.103553*

[7] Bapte PS, Pansambal SS, Bardapurkar PP, Ghotekar S, & Borgave SS. Eco-friendly synthesis of ZnO nanoparticles using pomegranate leaves and its antimicrobial, antioxidant, anticancer, angiogenic activity, and toxicity assessment. *Results in Chemistry. 2025;16:102460.https://doi.org/10.1016/j.rechem.2025 .102460*

[8] Echeverri K & Zayas RM. Regeneration: From cells to tissues to organisms. *Developmental biology. 2018;433(2):109–110. https://doi.org/10.1016/j.ydbio .2017.12.005*

[9] Shaik F. Advancements In Regenerative Medicine: Present Approaches, Emerging Strategies, And Future Perspectives. *International Journal of Advanced Research. 2024;12: 265-274. 10.21474/IJAR01 /19638.*

[10] Tatullo M, Zavan B & Piattelli A. Critical Overview on Regenerative Medicine: New Insights into the Role of Stem Cells and Innovative Biomaterials. *International journal of molecular sciences,2023;24(9):7936. https://doi.org/10.3390 /ijms24097936*

[11] Jourdan J, Riesch R & Cunze S. Off to new shores: Climate niche expansion in invasive mosquitofish (*Gambusia* spp.). *Ecology and evolution. 2021;11(24): 18369–18400. https://doi.org/10.1002/ece3.8427*

[12] Johnson DA & Denton TE. Caudal Fin Regeneration in the Mosquito Fish, *Gambusia affinis*: An in Vivo System for the Study of Ultraviolet Radiation Damage. *Radiat Res. 1977;*69(2): 241–247. https://doi.org/10.2307/3574432

[13] Borgave S, Ghule N & Naikwadi K. Effect of Glucose on Tail Fin Regeneration in *Gambusia affinis*. *Trends in Life Sciences. 2016; 5:273.*

[14] MacDonald RS. The role of zinc in growth and cell proliferation. *The Journal of nutrition, 2000; 130(5S Suppl):1500S–8S. https://doi.org/10.1093/jn /130.5. 1500S*

[15] Mandal AK, Katuwal S, Tettey F, Gupta A, Bhattarai S, Jaisi S, Bhandari DP, Shah AK, Bhattarai N & Parajuli N. Current Research on Zinc Oxide Nanoparticles: Synthesis, Characterization, and Biomedical Applications. *Nanomaterials (Basel, Switzerland). 2022; 12(17): 3066. https://doi.org /10.3390 /nano12173066*

[16] Nosrati R, Kheirouri S, Ghodsi R, Ojaghi H. The effects of zinc treatment on matrix metalloproteinases: A systematic review. *Journal of Trace Elements in Medicine and Biology. 2019; 56: 107-115 https://doi. org/10.1016/j.jtemb.2019.08.001*

[17] Wiesmann N, Mendler S, Buhr CR, Ritz U, Kämmerer, PW & Brieger J. Zinc Oxide Nanoparticles Exhibit Favorable Properties to Promote Tissue Integration of Biomaterials. *Biomedicines. 2021; 9(10): 1462. https://doi.org/10.3390/biomedicines9101462*

[18] Sirelkhatim A, Mahmud S, Seeni A, Kaus NHM, Ann LC, Bakhori SKM, Hasan H & Mohamad D. Review on Zinc Oxide Nanoparticles: Antibacterial Activity and Toxicity Mechanism. *Nano-micro letters. 2015; 7(3): 219–242. https://doi.org/10.1007/s40820-015-0040-x*

[19] Uberoi A, McCready-Vangi A & Grice EA. The wound microbiota: microbial mechanisms of impaired wound healing and infection. *Nat Rev Microbiol. 2024;22:507–521https://doi.org/10.1038/s41579-024-01035-z*

[20] Wei X, Liu Q, Wang Z. *et al.* Zinc oxide nitrogen oxide crosses the placental barrier and induces fetal malformations through activation of the Wnt/β-catenin signaling pathway. *J Nanopart Res. 2024;26: 161 https://doi.org/10.1007/s11051-024-06079-z*

[21] Shen C, James SA, deJonge MD, Turney TW, Wright PF & Feltis BN. Relating cytotoxicity, zinc ions, and reactive oxygen in ZnO nanoparticle-exposed human immune cells. *Toxicological sciences: an official journal of the Society of Toxicology. 2013;136(1): 120–130. https://doi.org/10.1093/toxsci/kft187*

[22] Wu F, Harper BJ & Harper SL. Comparative dissolution, uptake, and toxicity of zinc oxide particles in individual aquatic species and mixed populations. *Environmental toxicology and chemistry*. 2019;*38*(3): 591–602. https://doi.org/10.1002/etc.4349