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RESEARCH ARTICLE

Green synthesis iron oxide nanoparticles (Fe@AV NPs) induce developmental toxicity and anxiety-like behavior in zebrafish embryo-larvae

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ABSTRACT

The synthesis of nanoparticles and the usage areas of these nanoparticles show a rapid increase. In addition to the beneficial use of nanoparticles, their toxic effects cannot be ignored. In our study, iron oxide nanoparticle (Fe@AV NPs) (mean size: 20.852 nm) was synthesized from *Aloe vera* plant and the developmental toxicity of zebrafish was investigated. Zebrafish embryo-larvae were treated with different concentrations of Fe@AV NPs (1, 10, and 50 mg/L) starting at 4 hours after fertilization and continuing until 96 hours, and different developmental parameters (such as survival rate, hatchability rates, malformations, and behavior) were examined. In our study, it was determined that Fe@AV NPs caused developmental toxicity in zebrafish embryos depending on the dose increase. More than 60% died at 96 hours, especially in the highest (50 mg/L) application group. It was observed that Fe@AV NPs decreased and delayed the success of exiting the chorion depending on the dose increase, and caused various morphological abnormalities (like pericardial edema, tail deformation, and scoliosis) in all application groups except the lowest application group (1 mg/L). While 10 mg/L Fe@AV NPs caused sleep-like behaviors during the daytime by decreasing the daytime motility of the larvae, it caused hyperactivity by increasing their nocturnal motility. The results of thigmotaxis, which is an anxiety parameter, were found to increase anxiety at 10 mg/L Fe@AV NPs exposure.Our findings showed that Fe@AV NPs synthesized from *Aloe vera* plant have in vivo toxicity and their use at concentrations lower than 1 mg/L can be safe in environmental and medical applications.

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Introduction

Synthesis of nanoparticles (NPs) are growing unstoppably in many fields. However, nanotoxicological research is still in its infancy, with the impact of nanoparticles on the environment (Elsaesser & Howard, 2012). Iron oxide nanoparticles (IONPs) are now widely used in drug delivery, water remediation, and medical applications (Choi et al., 2019; Belda Marín et al., 2021; Martin et al., 2022).

IONPs are used in remediation and removal methods against pollutants in aquatic environments (Baruah et al., 2020; Kamath et al., 2020; Monje et al., 2022). This situation has made it important and necessary to investigate the toxic effects of IONPs on aquatic organisms. The toxicity of IONPs in some aquatic organisms is of concern (Sheel et al., 2020; Khoei, 2021; Paulpandian et al., 2022). It has been reported that when carp (*Cyprinus carpio*) is exposed to IONP (size: 20–40 nm) for 21 days, nanoparticles accumulate in the fish liver and intestines and show immunotoxicity in these tissues (Khoei, 2021). Similarly, guppy fish (*Poecilia reticulate*) prolonged (21 days) exposure to IONPs showed high histopathological indices in liver tissues due to circulatory disorders and inflammatory responses (Qualhato et al., 2018). Studies in trout showed that acetylcholinesterase (AChE) activity was decreased in the brain tissues of fish exposed to iron nanoparticles (Fe_3O_4-MNPs) for 96 hours. In addition, it has been reported that there is an increase in lipid peroxidation indicator (MDA values) and the levels of some biochemical parameters (TNF-α, 8-OHdG, and caspase-3) (Ucar et al., 2022). In addition, exposure of zebrafish (*Danio rerio*) embryos to iron nanoparticles (20–30 nm) for 5 days increased mortality, delayed hatchability, and impaired motor abilities (Huang et al., 2019). Still, after zebrafish embryos were statically and semi-statically exposed to IONPs at different doses (21.4 ± 0.39 nm; 0.3-10 mg/L) for 144 hours, it was observed that nanoparticles accumulated in the chorion of embryos and the digestive system of larvae blood accumulation and pericardial edema were observed (Pereira et al., 2020).

It is thought that the natural toxicities of nanoparticles can be used to improve existing cancer treatments through the production of iron nanoparticles, reactive oxygen species (ROS) (Könczöl et al., 2013; Hauser et al., 2016). This idea led to the synthesis of nanoparticles with less toxicity. Green synthesis nanoparticle production offers significant advantages over other nanoparticle synthesis methods as they are cost-effective and do not require the use of highly toxic chemicals (Murugan et al., 2018). Green synthesis nanoparticles are mostly synthesized using plant extracts (Rabiee et al., 2020; Paiva-Santos et al., 2021; Perumal et al., 2021; Kokturk et al., 2022, 2023; Sabeena et al., 2022). Some studies have shown that green-synthesized nanoparticles are less toxic than chemically synthesized NPs (Verma et al., 2020; Anila et al., 2021; Kokturk et al., 2022; Sabeena et al., 2022).

Aloe vera is a perennial and succulent plant whose main components are *Aloe vera* gel and latex (Viljoen et al., 2001). It has been reported that there are many bioactive components in the leaf of the plant (Minjares-Fuentes et al., 2018; Rehman et al., 2019). It has been reported that these components of the plant cause antibacterial, anti-inflammatory, and anticancer effects (Fehrmann-Cartes et al., 2019; Majumder et al., 2019; Medina-Cruz et al., 2021). However, it is very important to investigation the content of some phenolic compounds in *Aloe vera* leaf extract due to their cytotoxicity, and carcinogenicity (Guo & Mei, 2016). Because some studies have shown that *Aloe vera* plant has a toxic effect on living things (Lee et al., 2014; Nalimu et al., 2021; Amri et al., 2022).

In this context, our study aimed to determine the effects of green synthesis iron oxide nanoparticles (Fe@AV NPs) synthesized from *Aloe vera* plant on developmental changes (survival rate, larval hatching rate, and malformations) and behavior (locomotor activity and thigmotaxis) in zebrafish embryos and larvae.

Material and Methods

Chemicals and Instruments

Chemicals were purchased commercially from Merck. In the structural characterization of the samples, SEM (Hitachi Regulus 8230 FE-SEM, 10kV, 100X, 200X), TEM (Hitachi HT7800 TEM, 100Kv, 100000X), FT-IR (Spectrum Two FT-IR Spectrometer), XRD (Panalytical Empyrian, 2θ angle between 10-90o) brand devices were used

Preparation of Aloe vera

Fully grown fresh leaves of *Aloe vera* were washed with ultrapure water. The *Aloe vera* plant was kept in a low temperature (37.5°C) oven for five days. Then the dried plant was ground with the help of a blender. The ground plant was taken up in 100 ml of ethanol and boiled at about 80°C. After boiling, filtration was done. The resulting mixture was filtered using filter paper (Whatman: 1) after cooling. The resulting liquid mixture was stored at +4°C to be used in studies (Ozturk et al., 2022) (Figure 1).

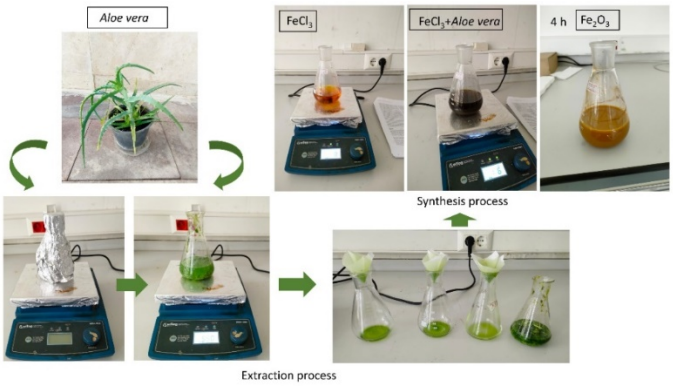

Figure 1. Steps of green synthesis of Fe@AV NPs using leaf extract of *Aloe vera*

Green Synthesis of Fe@AV NPs

0.6 M 100 ml $FeCl_3$ solution was prepared with ultrapure water. The prepared solution was stirred for 15 minutes to completely dissolve in the magnetic stirrer. Then it was mixed with 80 ml $FeCl_3$ solution and 20 ml *Aloe vera*. The resulting solution was left to cool for 2 hours for centrifugation after being subjected to a heating-shaking process in a closed water bath for 4 hours at 60°C. Centrifugation was continued at 4000 rpm for 5 minutes. During the process, the solid material was washed twice with ultrapure water and once with ethyl alcohol and kept in an oven at 60°C for 24 h. Finally, the characterization of the obtained solid material was started (Rautela & Rani, 2019) (Figure 1).

Zebrafish Breeding and Spawning

The adult fish from which the embryos used in our study were obtained, the AB striped zebrafish (*Danio rerio*) were obtained from Oregon State University. Zebrafish are reared at 28±1°C with a photoperiod of 14 h light and 10 h dark, with water circulation. Adult zebrafish are fed twice a day (artemia and flake food). Fertilized eggs were obtained from healthy adult zebrafish, as was done in previous studies (Sulukan et al., 2017).

Fe@AV NPs Treatment to Zebrafish Embryos-larvae

The iron oxide nanoparticle concentrations were derived from previous iron nanoparticle studies (Zhang et al., 2015; Pereira et al., 2020). Stock solution of nanoparticles was prepared with ultrapure water and kept in the sonicator for 20 minutes. Three different concentrations (1, 10, and 50 mg/L) were prepared with E3 solution (5 mM NaCI, 0.17 mM KCI, 0.33 mM $CaCI_2$, 0.33 mM $MgSO_4$, %0.01 methylene blue) from the stock solution and 40 embryos were used in each treatment group. The experiment was performed in three replications. Embryos were exposed to nanoparticles 4 hours after fertilization and exposure continued for 96 hours. In the experiment, solutions were renewed every 24 hours (semi-static exposure) (von Hellfeld et al., 2020). All developmental changes of embryos and larvae were examined at 24, 48, 72, and 96 hpf (OECD, 2013).

Developmental Toxicity of Fe@AV NPs (survival rate, hatchability rates, and malformation rate)

After the embryos and larvae were exposed to different concentrations of Fe@AV NPs, the survival rate was in the range of 4-96 hpf and the morphological changes were examined under the microscope and photographed. Hatchability rates was carried out between 48-96 hpf (Köktürk et al., 2022).

Effects of Fe@AV NP on the Behavior of Zebrafish Larvae (thigmotaxis and locomotor activity analyzes)

Locomotor activity was made using 16 (2 replicates) 96 hpf larvae randomly selected from each group and placed in a plate. The plate was placed on the DanioVision (Noldus) instrument set at 28.5°C and the behavior of the larvae was recorded for 50 min. (dark and light cycles). Motion records dark and light cycles data and total distance analysis of each larva were evaluated by EthoVision (Noldus) software (Kiziltan et al., 2022).

Thigmotaxis (anxiety index) was determined in larvae by making minor changes according to the method described by Schnörr et al. (2012). The purpose of the thigmotaxis analysis is to determine the larvae in the transition from light to dark by measuring the total distance traveled in the outer region (2.25 mm outer) of the wells in the plates by the ratio between the entire test area. As with locomotor activity, the evaluation of the behavior of their larvae for thigmotaxis analysis was performed with the EthoVision software (Baran et al., 2020).

Data Analysis

Developmental toxicity (survival rate, hatchability rates, and malformations) and behavior parameters were analyzed using ANOVA followed by Tukey post hoc testing. GraphPad Prism 8 software was used for data analysis and preparation of graphs. Results are given as mean±SD. Statistical significance (****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ compared to control) or ns: not significant

Results

Characterization of green synthesis of Fe@AV NPs (FT-IR, XRD, FE-SEM, EDS, and TEM)

FT-IR provides information on the vibrational properties of chemical functional groups. FT-IR spectra of the samples (Perk Marble Spectrum FTIR spectra) were obtained using FT-IR Spectrometers using KBr pellets, in the range of 4000 to 400 cm^{-1} with 4 cm^{-1} resolution. In the spectra of Fe@AV NPs, peaks of 3339 cm^{-1}, 1624 cm^{-1}, 1382 cm^{-1}, 865 cm^{-1}, 670 cm^{-1}, and 496 cm^{-1} were obtained. The peaks obtained in the spectral regions are 3339 cm^{-1} hydroxyl group (OH), 1624 cm^{-1} carbonyl group (O=C), 1382 cm^{-1} aliphatic amine (C-N), 865 cm^{-1} and 670 cm^{-1} (Fe-O), was paired with the 496 cm^{-1} alkane group (C-H) (Herlekar et al., 2014; Xiao et al., 2016; Hussain et al., 2016; Abid et al., 2022) (Figure 2).

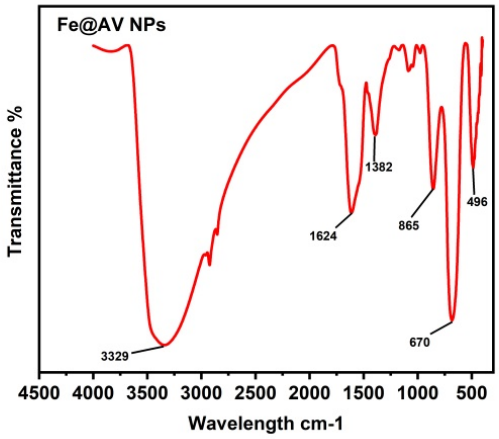

Figure 2. Image of FT-IR spectra of Fe@AV NPs

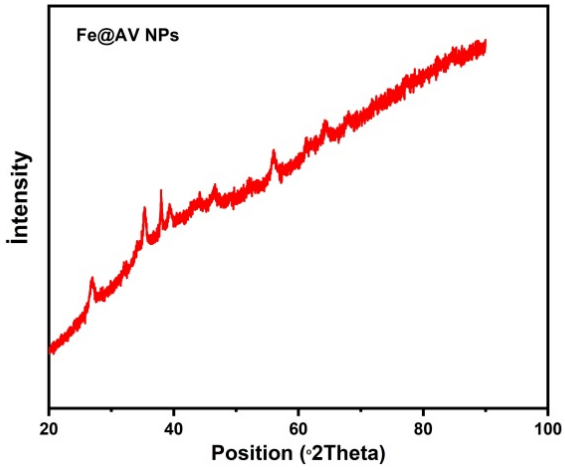

Figure 3. XRD spectra of Fe@AV NPs

With the aid of X-ray diffraction (XRD), the crystal size of the sample was evaluated. Iron oxide formation was confirmed using XRD (peaks obtained at 26.39, 34.89, 37.57, 45.80) (Herlekar et al., 2014; Hussain et al., 2016) (Figure 3).

Normal SEM imaging cannot be performed on iron-bound nanoparticles obtained by green synthesis. Because the magnetic field created by SEM is not preferred because it disturbs Fe-bound nanoparticles and creates agglomeration and stacking. Therefore, the surface morphology and size of the nanoparticles were determined with FE-SEM (Field Emission Scanning Electron Microscopes) and TEM devices. In addition, their elemental compositions were determined with the help of EDS. Figure 4 represents the SEM image of iron-containing nanoparticles with different shapes such as irregular hemispherical and cubic (Mahdavi et al., 2013; Herlekar et al., 2014; Majumder et al., 2019; Abid et al., 2022).

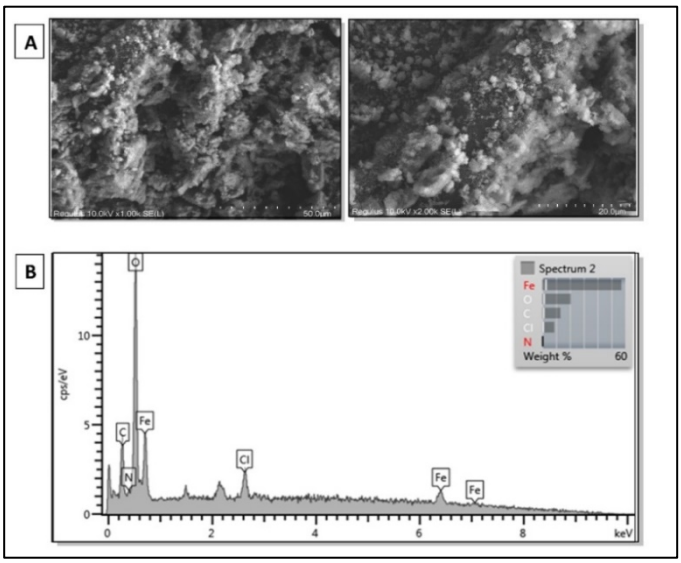

Figure 4. FE-SEM (A) and EDS (B) image showing the morphology of the biosynthesized Fe@AV NPs

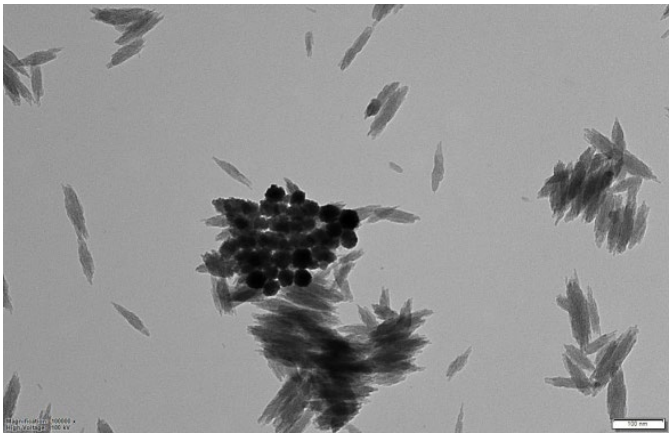

Figure 5. TEM image showing the morphology of Fe@AV NPs synthesized by green synthesis

Figure 5 shows the TEM image of magnetite nanoparticles synthesized using *Aloe vera* extract. The figure shows the image of some spherical or hemispherical and cylindrical

nanoparticles. In addition, the average size of the obtained cylindrical magnetic particles was determined as 20.852 nm from the image program. However, some clusters are noticeable in these images. These clusters sometimes form at the first moment and sometimes within a few days (Herlekar et al., 2014; Xiao et al., 2016; Majumder et al., 2019).

Developmental toxicity of Fe@AV NPs

The survival rate (24-96 hpf) of zebrafish embryos-larvae exposed to Fe@AV NPs is shown in Figure 6. There was a significant dose-dependent difference between the exposed groups (1, 10, and 50 mg/L) compared to the control (Figure 6). The survival rate was decreased in the Fe@AV NPs groups compared to the control (96.7%), and the survival rates for the 1, 10, and 50 mg/L groups were determined as 90.8%, 81.7% and 39.2%, respectively.

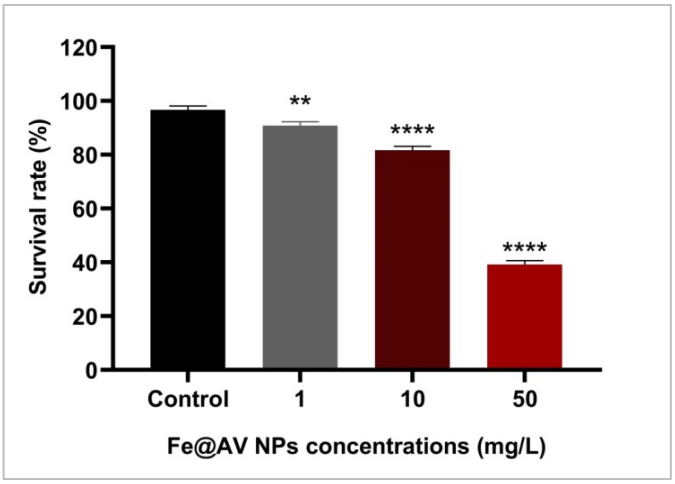

Figure 6. The survival rate of zebrafish embryo-larvae at different concentrations of Fe@AV NPs

Zebrafish embryo and larvae hatching at 48, 72, and 96 hpf were concentration-dependently delayed by Fe@AV NPs (Figure 7). In our study, while the hatching rate of the 48 hpf control group was 63.33%, the hatching rate of the 1, 10, and 50 mg/L concentration groups was 40.83%, 36.67%, and 21.67%, respectively, and significantly decreased compared to the control (Figure 7). Similarly, when the control and Fe@AV NPs application groups were compared at 72 and 96 hours, it was found that there was a significant difference (Figure 7).

The results for the morphological changes showed that there were phenotype distortions in the 1 mg/L concentration group, but there was no significant difference compared to the control group (Figures 8-9). However, phenotype changes such as pericardial edema, tail deformation, and scoliosis in the 10 (13.29%) and 50 (36.11%) mg/L groups of Fe@AV NPs were significantly significant with the control group (Figures 8-9).

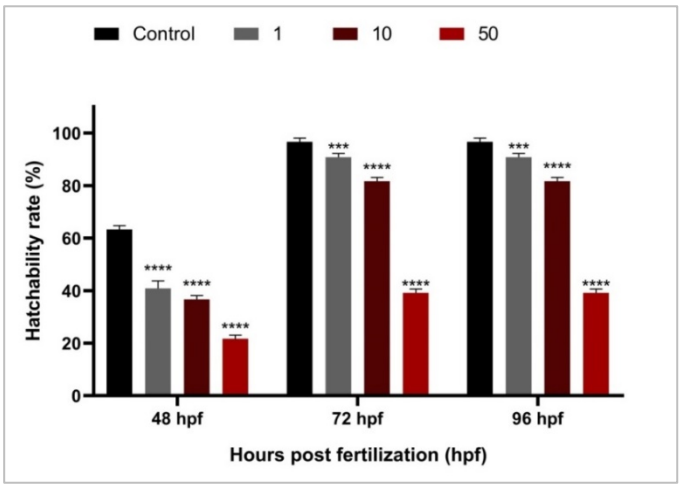

Figure 7. Concentration- and time-dependent hatchability rates for Fe@AV NPs in zebrafish embryos

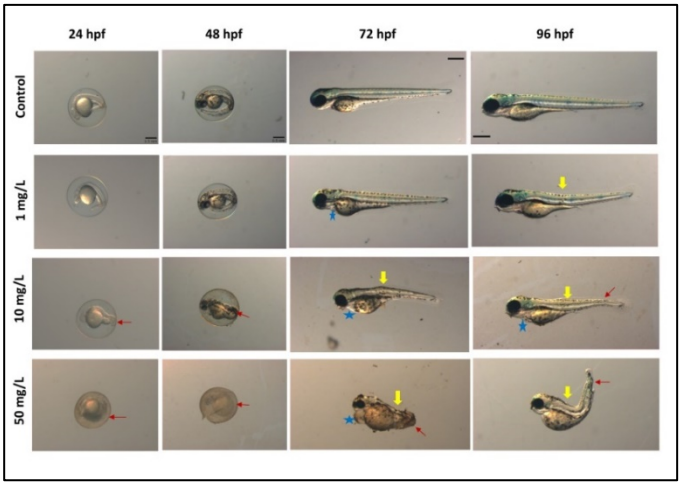

Figure 8. Visual inspection of embryo and larvae at 24, 48, 72, and 96 hpf. Scale bar: 0.5mm

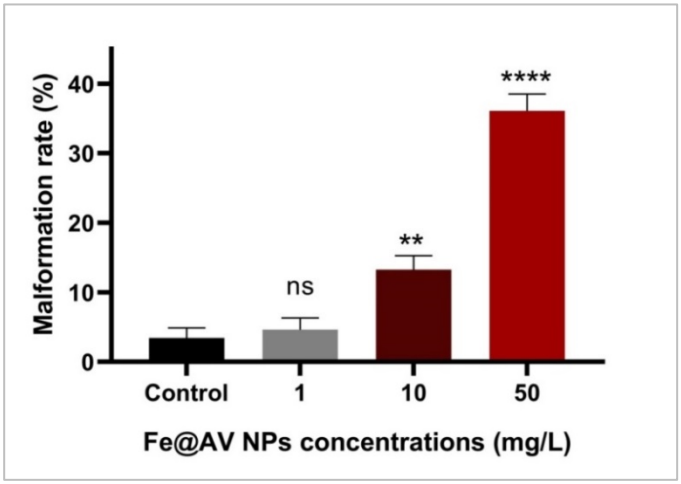

Figure 9. Malformation rate of zebrafish embryo-larvae at different concentrations of Fe@AV NPs

Effects of Fe@AV NPs on the Behavior of Zebrafish larvae

In our study, because the larvae were completely immobile in the 50 mg/L application group at the 96 hours, behavioral testing was not performed in these groups. Thigmotaxis and locomotor activity analyzes were performed to detect the change in behavior of zebrafish larvae exposed to Fe@AV NPs at concentrations of 1 and 10 mg/L for 96 hours (Figure 10). When the change in the distance traveled was examined, it was determined that especially 10 mg/L Fe@AV NPs caused sleep-like behaviors in the larvae during the day by decreasing their daytime mobility and causing hyperactivity by increasing the nighttime mobility (Figure 10A). When the results of thigmotaxis, which is an anxiety and anxiety parameter, were analyzed, it was found that it increased and induced anxiety at 10 mg/L Fe@AV NPs exposure (Figure 10B).

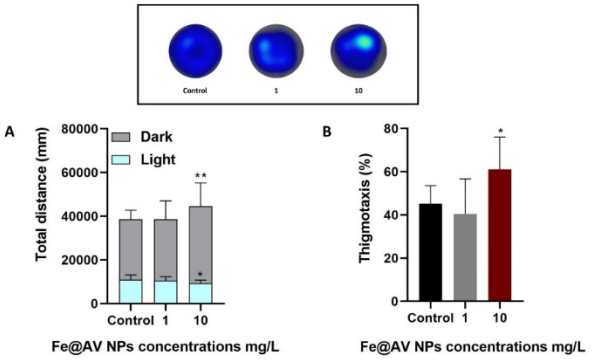

Figure 10. Dark/light locomotor activity (A) and anxiety like behavior (thigmotaxis) (B) of 96 hpf zebrafish larvae.

Discussion

It is thought that the cause of death of embryos in the chorion is that nanoparticles agglomerate on the chorion, increasing the chorion thickness, which in turn blocks the nutrient and oxygen exchange (de Medeiros et al., 2021). Aggregation in iron oxide nanoparticles indicates that these materials tend to accumulate near the bottom and in the sediment rather than in the water column This may make benthic organisms such as zebrafish embryos potential targets of nanoparticles in nature (Zhu et al., 2012). In our study, it was determined that Fe@AV NPs nanoparticles caused death in embryos and larvae depending on the dose increase. It can be said that Fe@AV nanoparticles cause death by accumulating in the chorion membrane of embryos and the pericardial region of larvae with their aggregation tendency. In addition, it is thought that toxic phenolic compounds in *Aloe vera* plant, which is used for the green synthesis reaction, may affect the survival rate of embryos and larvae (Guo & Mei, 2016).

It is known that the size of some iron nanoparticles is smaller than the chorion pore size (500–700 nm), which exposes zebrafish embryos to nanoparticles and even delays their exit from the chorion by clogging the chorion pores (Rawson et al., 2000; Pereira et al., 2020; de Almeida et al., 2021). This may lead to pre-hatching hypoxia changes by closing the chorionic pore channels and reducing gas exchange of embryos (Malafaia et al., 2020). It is also known that metallic nanoparticles adsorb to the surface of the chorion by forming complexes with the thiol group of chorionic proteins (Auffan et al., 2014). The green synthesis iron oxide nanoparticle (Fe@AV NPs) synthesized in our study was found to be large enough to easily pass through the chorion channels and by adhering to the chorion surface, delaying the hatchability of embryos from the chorion in all application groups. It has also been reported that iron oxide nanomaterials inhibit the activity of hatching enzymes and prevent zebrafish embryos from hatching (Zheng et al., 2023).

It has been shown that excessive and sustained iron overload can cause heart damage through ferroptosis (Fang et al., 2019). Again, some studies have reported that nanomaterials (nFexOy) formed with the element iron cause pericardial edema and collection of blood in the heart region in zebrafish embryos and larvae (Thirumurthi et al., 2022; Zheng et al., 2023). In our study, similar to the above studies, it was determined that exposure to Fe@AV NPs caused pericardial edema by affecting cardiac development in zebrafish embryos and larvae.

Swimming movements in larvae constitute behavioral and physiological integrity. Monitoring for differences in swimming movements is an additional and important approach for assessing toxic effects compared to other developmental parameters such as morphological changes and survival rates (Oliveira et al., 2020). In zebrafish larvae, locomotor behaviors are initiated and controlled by the nervous system (Drapeau et al., 2002). Therefore, locomotor activity is used as a sensitive indicator for changes in nerve development in abnormal situations (Liu et al., 2022). Many studies have determined that locomotor activity is somewhat impaired by concentration in zebrafish larvae exposed to nanoparticles such as TiO_2, Au, ZnO and SiO_2 (Duan et al., 2013; Chen et al., 2014; Hu et al., 2017; Xue et al., 2018). According to the results of thigmotaxis and dark/light locomotor activity in our study, it was observed that 10 mg/L Fe@AV NPs caused anxiety-like behaviors in zebrafish larvae and increased locomotor activity,

especially in the dark phase. These observations suggest that Fe@AV NPs exposure may have impaired the coordination between the nervous system and muscle connections (Basnet et al., 2019). It has been reported that light-dark transition increases locomotor activity in zebrafish larvae, while dark-light transition decreases locomotor activity. In zebrafish, increased locomotor activity is related to brain functions and nervous system development, and increased anxiety in larvae is associated with these conditions (Sulukan et al., 2023).

Conclusion

Our results showed that green synthesis iron oxide nanoparticles cause toxicity in zebrafish embryos and larvae. Fe@AV nanoparticles decreased the survival rate and hatchability in zebrafish embryos and larvae, while malformation rates increased.

In addition, it was determined that green synthesis Fe@AV NPs caused hyperactivity and increased anxiety in larvae. Compared to other chemical methods, green synthesis nanoparticles are considered to be less toxic. However, our study showed that green synthesis nanoparticles (Fe@AV NPs) can cause different toxicities depending on the *Aloe vera* used in synthesis. Therefore, it is important to determine safe dose ranges of green synthesis nanoparticles for all living things in the ecosystem.

Acknowledgments

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Compliance With Ethical Standards

Authors' Contributions

MK, AY, & ES: Designed the study.
MK: Wrote the first draft of the manuscript.
MK, AY, and ES: analyses were organized
MK, ES: Performed and managed statistical analyses.
All authors read and approved the final manuscript.

Conflict of Interest

The author declares that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required. Zebrafish larvae younger than 5 days old were used in the study. Therefore, the work does not require any license (Directive 86/609/EEC and EU Directive 2010/63/EU). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Data Availability Statements

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

Abid, M. A., Kadhim, D. A., & Aziz, W. J. (2022). Iron oxide nanoparticle synthesis using trigonella and tomato extracts and their antibacterial activity. *Materials Technology*, *37*(8), 547-554. https://doi.org/10.1080/10667857.2020.1863572

Amri, A., Bouraoui, Z., Balbuena-Pecino, S., Capilla, E., Gharred, T., Haouas, Z., Guerbej, H., Hosni, K., Navarro, I., & Jebali, J. (2022). Dietary supplementation with *Aloe vera* induces hepatic steatosis and oxidative stress together with a disruption of cellular signaling pathways and lipid metabolism related genes' expression in gilthead sea bream (*Sparus aurata*). *Aquaculture*, *559*, 738433. https://doi.org/10.1016/j.aquaculture.2022.738433

Anila, P. A., Keerthiga, B., Ramesh, M., & Muralisankar, T. (2021). Synthesis and characterization of palladium nanoparticles by chemical and green methods: A comparative study on hepatic toxicity using zebrafish as an animal model. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *244*, 108979. https://doi.org/10.1016/j.cbpc.2021.108979

Auffan, M., Matson, C. W., Rose, J., Arnold, M., Proux, O., Fayard, B., Liu, W., Chaurand, P., Wiesner, M. R., Bottero, J. Y., & Di Giulio, R. T. (2014). Salinity-dependent silver nanoparticle uptake and transformation by Atlantic killifish (*Fundulus heteroclitus*) embryos. *Nanotoxicology*, *8*(sup1), 167-176. https://doi.org/10.3109/17435390.2013.869627

Baran, A., Sulukan, E., Türkoğlu, M., Ghosigharehagaji, A., Yildirim, S., Kankaynar, M., Bolat, I., Kaya, M., Topal, A., & Ceyhun, S. B. (2020). Is sodium carboxymethyl cellulose (CMC) really completely innocent? It may be triggering obesity. *International Journal of Biological Macromolecules*, *163*, 2465-2473. https://doi.org/10.1016/j.ijbiomac.2020.09.169

Baruah, J., Chaliha, C., Kalita, E., Nath, B. K., Field, R. A., & Deb, P. (2020). Modelling and optimization of factors influencing adsorptive performance of agrowaste-derived Nanocellulose Iron Oxide Nanobiocomposites during remediation of Arsenic contaminated groundwater. *International Journal of Biological Macromolecules*, *164*, 53-65. https://doi.org/10.1016/j.ijbiomac.2020.07.113

Basnet, R. M., Zizioli, D., Taweedet, S., Finazzi, D., & Memo, M. (2019). Zebrafish larvae as a behavioral model in neuropharmacology. *Biomedicines*, *7*(1), 23. https://doi.org/10.3390%2Fbiomedicines7010023

Belda Marín, C., Egles, C., Humblot, V., Lalatonne, Y., Motte, L., Landoulsi, J., & Guénin, E. (2021). Gold, silver, and iron oxide nanoparticle incorporation into silk hydrogels for biomedical applications: elaboration, structure, and properties. *ACS Biomaterials Science & Engineering*, *7*(6), 2358-2371. https://doi.org/10.1021/acsbiomaterials.1c00441

Chen, T. H., Lin, C. C., & Meng, P. J. (2014). Zinc oxide nanoparticles alter hatching and larval locomotor activity in zebrafish (*Danio rerio*). *Journal of Hazardous Materials*, *277*, 134-140. https://doi.org/10.1016/j.jhazmat.2013.12.030

Choi, G. E., Kang, M. S., Kim, Y. J., Yoon, J. J., & Jeong, Y. I. (2019). Magnetically responsive drug delivery using doxorubicin and iron oxide nanoparticle-incorporated lipocomplexes. *Journal of Nanoscience and Nanotechnology*, 19(2), 675-679. https://doi.org/10.1166/jnn.2019.15910

de Almeida, V. O., Pereira, T. C. B., de Souza Teodoro, L., Escobar, M., Ordovas, C. J., Dos Santos, K. B., Weiler, J., Bogo, M. R., & Schneider, I. A. H. (2021). On the effects of iron ore tailings micro/nanoparticles in embryonic and larval zebrafish (*Danio rerio*). *Science of The Total Environment*, *759*, 143456. https://doi.org/10.1016/j.scitotenv.2020.143456

de Medeiros, A. M., Khan, L. U., da Silva, G. H., Ospina, C. A., Alves, O. L., de Castro, V. L., & Martinez, D. S. T. (2021). Graphene oxide-silver nanoparticle hybrid material: an integrated nanosafety study in zebrafish embryos. *Ecotoxicology and Environmental Safety*, *209*, 111776. https://doi.org/10.1016/j.ecoenv.2020.111776

Drapeau, P., Saint-Amant, L., Buss, R. R., Chong, M., McDearmid, J. R., & Brustein, E. (2002). Development of the locomotor network in zebrafish. *Progress in Neurobiology*, *68*(2), 85-111. https://doi.org/10.1016/S0301-0082(02)00075-8

Duan, J., Yu, Y., Shi, H., Tian, L., Guo, C., Huang, P., Zhou, X., Peng, S., & Sun, Z. (2013). Toxic effects of silica nanoparticles on zebrafish embryos and larvae. *PloS One*, *8*(9), e74606. https://doi.org/10.1371/journal.pone.0074606

Elsaesser, A., & Howard, C. V. (2012). Toxicology of nanoparticles. *Advanced Drug Delivery Reviews*, *64*(2), 129-137. https://doi.org/10.1016/j.addr.2011.09.001

Fang, X., Wang, H., Han, D., Xie, E., Yang, X., Wei, J., Gu, S., Gao, F., Zhu, N., Yin, X., Cheng, Q., Zhang, P., Dai, W., Chen, J., Yang, F., Yang, H. T., Linkermann, A., Gu, W., Min, J., & Wang, F. (2019). Ferroptosis as a target for protection against cardiomyopathy. *Proceedings of the National Academy of Sciences*, *116*(7), 2672-2680. https://doi.org/10.1073/pnas.1821022116

Fehrmann-Cartes, K., Coronado, M., Hernandez, A. J., Allende, M. L., & Feijoo, C. G. (2019). Anti-inflammatory effects of *Aloe vera* on soy meal-induced intestinal inflammation in zebrafish. *Fish & Shellfish Immunology*, *95*, 564-573. https://doi.org/10.1016/j.fsi.2019.10.075

Guo, X., & Mei, N. (2016). *Aloe vera*: A review of toxicity and adverse clinical effects. *Journal of Environmental Science and Health, Part C*, *34*(2), 77-96. https://doi.org/10.1080%2F10590501.2016.1166826

Hauser, A. K., Mitov, M. I., Daley, E. F., McGarry, R. C., Anderson, K. W., & Hilt, J. Z. (2016). Targeted iron oxide nanoparticles for the enhancement of radiation therapy. *Biomaterials*, *105*, 127-135. https://doi.org/10.1016/j.biomaterials.2016.07.032

Herlekar, M., Barve, S., & Kumar, R. (2014). Plant-mediated green synthesis of iron nanoparticles. *Journal of Nanoparticles*, *2014*, 140614. https://doi.org/10.1155/2014/140614

Hu, Q., Guo, F., Zhao, F., & Fu, Z. (2017). Effects of titanium dioxide nanoparticles exposure on parkinsonism in zebrafish larvae and PC12. *Chemosphere*, *173*, 373-379. https://doi.org/10.1016/j.chemosphere.2017.01.063

Huang, Z., Xu, B., Huang, X., Zhang, Y., Yu, M., Han, X., Song, L., Xia, Y., Zhou, Z., Wang, X., Chen, M., & Lu, C. (2019). Metabolomics reveals the role of acetyl-l-carnitine metabolism in γ-Fe_2O_3 NP-induced embryonic development toxicity via mitochondria damage. *Nanotoxicology*, *13*(2), 204-220. https://doi.org/10.1080/17435390.2018.1537411

Hussain, I., Singh, N. B., Singh, A., Singh, H., & Singh, S. C. (2016). Green synthesis of nanoparticles and its potential application. *Biotechnology Letters*, *38*(4), 545-560. https://doi.org/10.1007/s10529-015-2026-7

Kamath, V., Chandra, P., & Jeppu, G. P. (2020). Comparative study of using five different leaf extracts in the green synthesis of iron oxide nanoparticles for removal of arsenic from water. *International journal of phytoremediation*, *22*(12), 1278-1294. https://doi.org/10.1080/15226514.2020.1765139

Khoei, A. J. (2021). Evaluation of potential immunotoxic effects of iron oxide nanoparticles (IONPs) on antioxidant capacity, immune responses and tissue bioaccumulation in common carp (*Cyprinus carpio*). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *244*, 109005. https://doi.org/10.1016/j.cbpc.2021.109005

Kiziltan, T., Baran, A., Kankaynar, M., Şenol, O., Sulukan, E., Yildirim, S., & Ceyhun, S. B. (2022). Effects of the food colorant carmoisine on zebrafish embryos at a wide range of concentrations. *Archives of Toxicology*, *96*(4), 1089-1099. https://doi.org/10.1007/s00204-022-03240-2

Kokturk, M., Yıldırım, S., Atamanalp, M., Calimli, M. H., Nas, M. S., Bolat, I., Ozhan, G., & Alak, G. (2022). Assessment of oxidative DNA damage, apoptosis and histopathological alterations on zebrafish exposed with green silver nanoparticle. *Chemistry and Ecology*, *38*(7), 655-670. https://doi.org/10.1080/02757540.2022.2108808

Kokturk, M., Yıldırım, S., Calimli, M. H., Nas, M. S., Ibaokurgil, F., Ozhan, G., Atamanalp, M., & Alak, G. (2023). Perspective on green synthesis of RP-Pd/AC NPs: characterization, embryonic and neuronal toxicity assessment. *International Journal of Environmental Science and Technology*, *20*, 871-882. https://doi.org/10.1007/s13762-022-04005-1

Köktürk, M., Yildirim, S., Yiğit, A., Ozhan, G., Bolat, İ., Alma, M. H., Menges, N., Alak, G., & Atamanalp, M. (2022). What is the eco-toxicological level and effects of graphene oxide-boramidic acid (GO-ED-BA NP)?: In vivo study on Zebrafish embryo/larvae. *Journal of Environmental Chemical Engineering*, *10*(5), 108443. https://doi.org/10.1016/j.jece.2022.108443

Könczöl, M., Weiss, A., Stangenberg, E., Gminski, R., Garcia-Käufer, M., Gieré, R., Merfort, I., & Mersch-Sundermann, V. (2013). Cell-cycle changes and oxidative stress response to magnetite in A549 human lung cells. *Chemical Research in Toxicology*, *26*(5), 693-702. https://doi.org/10.1021/tx300503q

Lee, J., Lee, M. S., & Nam, K. W. (2014). Acute toxic hepatitis caused by an *Aloe vera* preparation in a young patient: a case report with a literature review. *The Korean Journal of Gastroenterology*, *64*(1), 54-58. https://doi.org/10.4166/kjg.2014.64.1.54

Liu, Y., Wang, Y., Li, N., & Jiang, S. (2022). Avobenzone and nanoplastics affect the development of zebrafish nervous system and retinal system and inhibit their locomotor behavior. *Science of The Total Environment*, *806*(Part 2), 150681. https://doi.org/10.1016/j.scitotenv.2021.150681

Mahdavi, M., Namvar, F., Ahmad, M. B., & Mohamad, R. (2013). Green biosynthesis and characterization of magnetic iron oxide (Fe_3O_4) nanoparticles using seaweed (*Sargassum muticum*) aqueous extract. Molecules, *18*(5), 5954-5964. https://doi.org/10.3390/molecules18055954

Majumder, R., Das, C. K., & Mandal, M. (2019). Lead bioactive compounds of *Aloe vera* as potential anticancer agent. *Pharmacological Research*, *148*, 104416. https://doi.org/10.1016/j.phrs.2019.104416

Malafaia, G., de Souza, A. M., Pereira, A. C., Gonçalves, S., da Costa Araújo, A. P., Ribeiro, R. X., & Rocha, T. L. (2020). Developmental toxicity in zebrafish exposed to polyethylene microplastics under static and semi-static aquatic systems. *Science of The Total Environment*, *700*, 134867. https://doi.org/10.1016/j.scitotenv.2019.134867

Martin, L. M., Sheng, J., Zimba, P. V., Zhu, L., Fadare, O. O., Haley, C., Wang, M., Phillips, T. D., Conkle, J., & Xu, W. (2022). Testing an iron oxide nanoparticle-based method for magnetic separation of nanoplastics and microplastics from water. *Nanomaterials*, *12*(14), 2348. https://doi.org/10.3390/nano12142348

Medina-Cruz, D., Vernet-Crua, A., Mostafavi, E., González, M. U., Martínez, L., Iii, A. A. D. J., Kusper, M., Sotelo, E., Gao, M., Geoffrion, L. D., Shah, V., Guisbiers, G., Cholula-Díaz, J. L., Guillermier, C., Khanom, F., Huttel, Y., García-Martín, J. M., & Webster, T. J. (2021). *Aloe vera*-mediated Te nanostructures: Highly potent antibacterial agents and moderated anticancer effects. *Nanomaterials*, *11*(2), 514. https://doi.org/10.3390/nano11020514

Minjares-Fuentes, R., Femenia, A., Comas-Serra, F., & Rodríguez-González, V. M. (2018). Compositional and structural features of the main bioactive polysaccharides present in the *Aloe vera* plant. *Journal of AOAC International*, *101*(6), 1711-1719. https://doi.org/10.5740/jaoacint.18-0119

Monje, D. S., Ruiz, O. S., Valencia, G. C., & Mercado, D. F. (2022). Iron oxide nanoparticles embedded in organic microparticles from Yerba Mate useful for remediation of textile wastewater through a photo-Fenton treatment: Ilex paraguariensis as a platform of environmental interest–Part 1. *Environmental Science and Pollution Research*, *29*, 57127-57146. https://doi.org/10.1007/s11356-022-19744-4

Murugan, K., Dinesh, D., Nataraj, D., Subramaniam, J., Amuthavalli, P., Madhavan, J., Rajasekar, A., Rajan, M., Thiruppathi, K. P., Kumar, S., Higuchi, A., Nicoletti, M., & Benelli, G. (2018). Iron and iron oxide nanoparticles are highly toxic to *Culex quinquefasciatus* with little non-target effects on larvivorous fishes. *Environmental Science and Pollution Research*, *25*(11), 10504-10514. https://doi.org/10.1007/s11356-017-0313-7

Nalimu, F., Oloro, J., Kahwa, I., & Ogwang, P. E. (2021). Review on the phytochemistry and toxicological profiles of *Aloe vera* and *Aloe ferox*. *Future Journal of Pharmaceutical Sciences, 7*(1), 145. https://doi.org/10.1186/s43094-021-00296-2

OECD. (2013) Test No. 236: Fish Embryo Acute Toxicity (FET) Test. OECD Guidelines for the Testing of Chemicals, Section, 2, 1-22.

Oliveira, E. M. N., Selli, G. I., von Schmude, A., Miguel, C. A. M. I. L. A., Laurent, S., Vianna, M. R. M., & Papaléo, R. M. (2020). Developmental toxicity of iron oxide nanoparticles with different coatings in zebrafish larvae. *Journal of Nanoparticle Research*, *22*(4), 87. https://doi.org/10.1007/s11051-020-04800-2

Ozturk, D., Ozguven, A., Yonten, V., & Ertas, M. (2022). Green synthesis, characterization and antimicrobial activity of silver nanoparticles using *Ornithogalum narbonense* L. *Inorganic and Nano-Metal Chemistry*, *52*(3), 329-341. https://doi.org/10.1080/24701556.2021.1978496

Paiva-Santos, A. C., Herdade, A. M., Guerra, C., Peixoto, D., Pereira-Silva, M., Zeinali, M., Mascarenhas-Melo, F., Paranhos, A., & Veiga, F. (2021). Plant-mediated green synthesis of metal-based nanoparticles for dermopharmaceutical and cosmetic applications. *International Journal of Pharmaceutics*, *597*, 120311. https://doi.org/10.1016/j.ijpharm.2021.120311

Paulpandian, P., Beevi, I. S., Somanath, B., Kamatchi, R. K., Paulraj, B., & Faggio, C. (2022). Impact of *Camellia sinensis* iron oxide nanoparticle on growth, hemato-biochemical and antioxidant capacity of blue gourami (*Trichogaster trichopterus*) fingerlings. *Biological Trace Element Research*, *201*(1), 412-424. https://doi.org/10.1007/s12011-022-03145-2

Pereira, A. C., Gonçalves, B. B., da Silva Brito, R., Vieira, L. G., de Oliveira Lima, E. C., & Rocha, T. L. (2020). Comparative developmental toxicity of iron oxide nanoparticles and ferric chloride to zebrafish (*Danio rerio*) after static and semi-static exposure. *Chemosphere*, *254*, 126792. https://doi.org/10.1016/j.chemosphere.2020.126792

Perumal, S., Gopal Samy, M. V., & Subramanian, D. (2021). Selenium nanoparticle synthesis from endangered medicinal herb (*Enicostema axillare*). *Bioprocess and Biosystems Engineering*, *44*(9), 1853-1863. https://doi.org/10.1007/s00449-021-02565-z

Qualhato, G., de Sabóia-Morais, S. M. T., Silva, L. D., & Rocha, T. L. (2018). Melanomacrophage response and hepatic histopathologic biomarkers in the guppy *Poecilia reticulata* exposed to iron oxide (maghemite) nanoparticles. *Aquatic Toxicology*, *198*, 63-72. https://doi.org/10.1016/j.aquatox.2018.02.014

Rabiee, N., Bagherzadeh, M., Kiani, M., & Ghadiri, A. M. (2020). *Rosmarinus officinalis* directed palladium nanoparticle synthesis: investigation of potential anti-bacterial, anti-fungal and Mizoroki-Heck catalytic activities. *Advanced Powder Technology*, *31*(4), 1402-1411. https://doi.org/10.1016/j.apt.2020.01.024

Rautela, A., & Rani, J. (2019). Green synthesis of silver nanoparticles from *Tectona grandis* seeds extract: characterization and mechanism of antimicrobial action on different microorganisms. *Journal of Analytical Science and Technology*, *10*(1), 5. https://doi.org/10.1186/s40543-018-0163-z

Rawson, D. M., Zhang, T., Kalicharan, D., & Jongebloed, W. L. (2000). Field emission scanning electron microscopy and transmission electron microscopy studies of the chorion, plasma membrane and syncytial layers of the gastrula-stage embryo of the zebrafish Brachydanio rerio: a consideration of the structural and functional relationships with respect to cryoprotectant penetration. *Aquaculture Research*, *31*(3), 325-336. https://doi.org/10.1046/j.1365-2109.2000.00401.x

Rehman, N. U., Al-Riyami, S. A., Hussain, H., Ali, A., Khan, A. A. L., & Al-Harrasi, A. (2019). Secondary metabolites from resins of *Aloe vera* and *Commiphora mukul* mitigate lipid peroxidation. *Acta Pharmaceutica*, *69*(3), 433-441. https://doi.org/10.2478/acph-2019-0027

Sabeena, G., Rajaduraipandian, S., Pushpalakshmi, E., Alhadlaq, H. A., Mohan, R., Annadurai, G., & Ahamed, M. (2022). Green and chemical synthesis of CuO nanoparticles: A comparative study for several in vitro bioactivities and in vivo toxicity in zebrafish embryos. *Journal of King Saud University-Science*, *34*(5), 102092. https://doi.org/10.1016/j.jksus.2022.102092

Schnörr, S. J., Steenbergen, P. J., Richardson, M. K., & Champagne, D. (2012). Measuring thigmotaxis in larval zebrafish. *Behavioural Brain Research*, *228*(2), 367-374. https://doi.org/10.1016/j.bbr.2011.12.016

Sheel, R., Kumari, P., Panda, P. K., Ansari, M. D. J., Patel, P., Singh, S., Kumari, B., Sarkar, B., Mallick, M. A., & Verma, S. K. (2020). Molecular intrinsic proximal interaction infer oxidative stress and apoptosis modulated in vivo biocompatibility of *P. niruri* contrived antibacterial iron oxide nanoparticles with zebrafish. *Environmental Pollution*, *267*, 115482. https://doi.org/10.1016/j.envpol.2020.115482

Sulukan, E., Baran, A., Kankaynar, M., Kızıltan, T., Bolat, İ., Yıldırım, S., Akgül Ceyhun, H., & Ceyhun, S. B. (2023). Global warming and glyphosate toxicity (II): Offspring zebrafish modelling with behavioral, morphological and immunohistochemical approaches. *Science of The Total Environment*, *856*(Part 1), 158903. https://doi.org/10.1016/j.scitotenv.2022.158903

Sulukan, E., Köktürk, M., Ceylan, H., Beydemir, Ş., Işik, M., Atamanalp, M., & Ceyhun, S. B. (2017). An approach to clarify the effect mechanism of glyphosate on body malformations during embryonic development of zebrafish (*Daino rerio*). *Chemosphere*, *180*, 77-85. https://doi.org/10.1016/j.chemosphere.2017.04.018

Thirumurthi, N. A., Raghunath, A., Balasubramanian, S., & Perumal, E. (2022). Evaluation of maghemite nanoparticles–induced developmental toxicity and oxidative stress in zebrafish embryos/larvae. *Biological Trace Element Research*, *200*(5), 2349-2364. https://doi.org/10.1007/s12011-021-02830-y

Ucar, A., Parlak, V., Ozgeris, F. B., Yeltekin, A. C., Arslan, M. E., Alak, G., Turkez, H., Kocaman, E. M., & Atamanalp, M. (2022). Magnetic nanoparticles-induced neurotoxicity and oxidative stress in brain of rainbow trout: Mitigation by ulexite through modulation of antioxidant, anti-inflammatory, and antiapoptotic activities. *Science of The Total Environment*, *838*(Part 1), 155718. https://doi.org/10.1016/j.scitotenv.2022.155718

Verma, S. K., Nisha, K., Panda, P. K., Patel, P., Kumari, P., Mallick, M. A., Sarkar, B., & Das, B. (2020). Green synthesized MgO nanoparticles infer biocompatibility by reducing in vivo molecular nanotoxicity in embryonic zebrafish through arginine interaction elicited apoptosis. *Science of The Total Environment*, *713*, 136521. https://doi.org/10.1016/j.scitotenv.2020.136521

Viljoen, A. M., Van Wyk, B. E., & Newton, L. E. (2001). The occurrence and taxonomic distribution of the anthrones aloin, aloinoside and microdontin in *Aloe*. *Biochemical Systematics and Ecology*, *29*(1), 53-67. https://doi.org/10.1016/s0305-1978(00)00024-7

von Hellfeld, R., Brotzmann, K., Baumann, L., Strecker, R., & Braunbeck, T. (2020). Adverse effects in the fish embryo acute toxicity (FET) test: A catalogue of unspecific morphological changes versus more specific effects in zebrafish (*Danio rerio*) embryos. *Environmental Sciences Europe*, *32*(1), 122. https://doi.org/10.1186/s12302-020-00398-3

Xiao, Z., Yuan, M., Yang, B., Liu, Z., Huang, J., & Sun, D. (2016). Plant-mediated synthesis of highly active iron nanoparticles for Cr (VI) removal: Investigation of the leading biomolecules. *Chemosphere*, *150*, 357-364. https://doi.org/10.1016/j.chemosphere.2016.02.056

Xue, W., Liu, Y., Zhang, N., Yao, Y., Ma, P., Wen, H., Huang, S., Luo, Y., & Fan, H. (2018). Effects of core size and PEG coating layer of iron oxide nanoparticles on the distribution and metabolism in mice. *International journal of Nanomedicine*, *13*, 5719. https://doi.org/10.2147/IJN.S165451

Zhang, Y., Zhu, L., Zhou, Y., & Chen, J. (2015). Accumulation and elimination of iron oxide nanomaterials in zebrafish (*Danio rerio*) upon chronic aqueous exposure. *Journal of Environmental Sciences*, *30*, 223-230. https://doi.org/10.1016/j.jes.2014.08.024

Zheng, N., Sun, X., Shi, Y., Chen, L., Wang, L., Cai, H., Han, C., Liao, T., Yang, C., Zuo, Z., & He, C. (2023). The valence state of iron-based nanomaterials determines the ferroptosis potential in a zebrafish model. *Science of The Total Environment*, *855*, 158715. https://doi.org/10.1016/j.scitotenv.2022.158715

Zhu, X., Tian, S., & Cai, Z. (2012). Toxicity assessment of iron oxide nanoparticles in zebrafish (*Danio rerio*) early life stages. *PLoS One*, *7*(9), e46286. https://doi.org/10.1371/journal.pone.0046286