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Behavioural and Neurodevelopmental Disruptions in Zebrafish Exposed to <20 nm Aluminium Oxide Nanoparticles

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KEYWORDS

Zebrafish, Aluminium oxide nanoparticles, Developmental toxicity, Neurobehavioral toxicity, Nanotoxicology, physico-chemical characterization

ABSTRACT:

Background: The rapid expansion of nanotechnology has led to the increased production and application of aluminum oxide nanoparticles (Al_2O_3 NPs) across biomedical, industrial, and environmental sectors. Despite their widespread use, concerns regarding their potential neurotoxic and developmental effects remain insufficiently addressed. Given their nanoscale size and enhanced reactivity, Al_2O_3 nanoparticles can interact with biological systems and potentially disrupt normal physiological processes. Therefore, evaluating their toxicity using relevant biological models is essential.

Objectives: This study aimed to investigate the physicochemical properties, developmental toxicity, and neurobehavioral effects of chemically synthesized Al_2O_3 nanoparticles (<20 nm) using zebrafish (Danio rerio) as a model organism.

Methods: Al_2O_3 nanoparticles were synthesized via chemical precipitation and characterized using UV–Visible spectroscopy, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), and inductively coupled plasma–atomic emission spectroscopy (ICP–AES). Developmental toxicity was assessed using the Fish Embryo Toxicity (FET) assay in accordance with OECD guidelines. Embryos were exposed to graded nanoparticle concentrations and monitored up to 120 hours post-fertilization for survival, hatching rate, heart rate, and morphological abnormalities. Neurobehavioral assessment was conducted in larvae at 5 days post-fertilization using automated video tracking, while adult zebrafish were subjected to chronic exposure (7–21 days) followed by behavioural analysis using locomotor tracking and spatial distribution (KDE) analysis.

Results: Characterization confirmed the successful synthesis of nanoscale, crystalline Al_2O_3 nanoparticles with defined morphology and elemental composition. Developmental toxicity studies revealed a clear dose-dependent effect, with concentrations ≥6 µg/mL inducing delayed hatching, reduced viability, bradycardia, and morphological abnormalities such as pericardial edema, yolk sac edema, and body curvature. Higher concentrations resulted in severe deformities and increased mortality. Behavioural analysis of larvae demonstrated reduced swimming speed, decreased exploratory behaviour, and increased inactivity, indicating impaired neuromotor function. In adult zebrafish, chronic exposure led to reduced locomotion, increased thigmotaxis, and anxiety-like behaviour. A non-monotonic dose-response pattern was observed, characterized by mild anxiety at lower concentrations, transient hyperactivity at intermediate levels, and progressive hypoactivity at higher concentrations.

Conclusion: Al_2O_3 nanoparticles induce significant developmental and neurobehavioral toxicity in zebrafish, affecting multiple biological endpoints. The findings highlight potential ecological and human health risks associated with nanoparticle exposure and emphasize the need for further mechanistic studies using molecular and multi-omics approaches. Additionally, the results support the development of safer-by-design nanomaterials to mitigate adverse biological effects

1. Introduction

The increasing prevalence of neurodevelopmental and cognitive disorders has emerged as a major global health concern, necessitating the development of reliable experimental models to investigate underlying mechanisms and environmental risk factors. Cognitive dysfunction, characterized by impairments in learning,

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memory, attention, and decision-making, is a hallmark of several neurological disorders, including Alzheimer's disease and related neurodegenerative conditions [1,2]. With rising life expectancy, the burden of such disorders is expected to increase significantly [3].

In addition to aging, environmental factors have been increasingly recognized as critical contributors to neurodevelopmental deficits. Among these, aluminum has gained attention due to its widespread industrial applications and potential neurotoxic effects [4,5]. Exposure to aluminum has been associated with oxidative stress, neuronal damage, and disruption of synaptic transmission, all of which may contribute to cognitive impairment [6,7].

Advancements in nanotechnology have led to the extensive production and application of aluminum-based nanomaterials, particularly aluminum oxide nanoparticles (Al_2O_3 NPs), in fields such as drug delivery, catalysis, cosmetics, and environmental remediation [8]. Due to their nanoscale size (<20 nm), high surface area, and enhanced reactivity, these nanoparticles can interact with biological systems and penetrate physiological barriers, including the blood–brain barrier [9,10]. While these properties make them valuable for technological applications, they also raise concerns regarding their potential toxicity[11].

Emerging evidence suggests that nanoparticle exposure can induce neurodevelopmental toxicity through mechanisms such as oxidative stress, apoptosis, neuroinflammation, and disruption of neurotransmitter systems [7,12]. Therefore, understanding the neurobehavioral effects of Al_2O_3 nanoparticles is essential for evaluating their safety and biological impact.

The zebrafish (Danio rerio) has become a widely accepted vertebrate model for studying developmental neurotoxicity due to its genetic similarity to humans, transparent embryos, rapid development, and well-characterized behavioural responses [13,14]. Zebrafish larvae, in particular, exhibit measurable locomotor and exploratory behaviours, making them suitable for neurobehavioral assessments [15].

This chapter investigates the physicochemical properties, developmental toxicity, and behavioural effects of chemically synthesized Al_2O_3 nanoparticles (<20 nm) using zebrafish as a model organism. By integrating nanoparticle characterization with biological endpoints, this study aims to provide a comprehensive understanding of their neurotoxic potential.

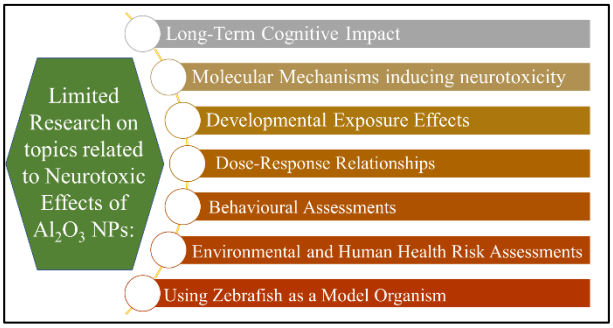

Figure 1: Research gap regarding Neurotoxic effects of Al_2O_3 NPs

Despite the widespread use of Al_2O_3 nanoparticles, their neurotoxic effects remain insufficiently understood, particularly in aquatic systems. Most existing studies focus on acute toxicity, with limited information on long-term developmental and cognitive outcomes [10; 11]. Furthermore, the molecular mechanisms underlying nanoparticle-induced neurotoxicity, including oxidative stress and neurotransmitter disruption, are not fully elucidated [7].

There is also a lack of comprehensive understanding of dose–response relationships and behavioural alterations, which are critical indicators of neurotoxicity. Additionally, the implications of Al_2O_3 nanoparticles for environmental and human health risk assessment remain underexplored [16]. Although zebrafish is a well-established model, its application in assessing neurobehavioral and developmental toxicity of Al_2O_3 nanoparticles is still limited. This study aims to address these gaps through an integrated toxicological approach.

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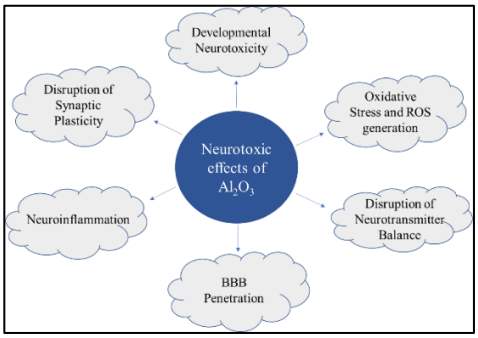

Figure 2 : Charts enlisting common Behavioural Assays for Zebrafish Figure & Neurotoxic effects of Al_2O_3 nanoparticles

A range of behavioural assays are employed in zebrafish research to evaluate cognitive function, anxiety, and social behaviour. The Y-maze and T-maze assess spatial learning and memory, while the Novel Tank Test and Light–Dark Preference test evaluate anxiety-like responses. The Novel Object Recognition test provides insights into cognitive function, and Social Preference assays assess social interactions. Collectively, these assays offer a comprehensive framework for studying neurobehavioral effects in zebrafish.

Al_2O_3 nanoparticles are associated with multiple mechanisms of neurotoxicity. These include oxidative stress and reactive oxygen species (ROS) generation, leading to cellular damage, as well as disruption of neurotransmitter balance, affecting neural signaling. Nanoparticles may also cross the blood–brain barrier, triggering neuroinflammation and impairing brain function. Additionally, they can interfere with synaptic plasticity and contribute to developmental neurotoxicity, ultimately affecting cognitive and behavioural outcomes.

2. Methods

2.1 Synthesis of Aluminium Oxide Nanoparticles

Chemical precipitation was used to create aluminum oxide nanoparticles (Al2O3 NPs), as previously reported [17]. To create a homogenous precursor solution, aluminum nitrate monohydrate ($Al(NO_3)_3 \cdot 9H_2O$) was dissolved in methanol with continuous stirring. Sodium hydroxide (NaOH) was used to progressively raise the pH of the solution to an alkaline level (pH ~12) in order to promote the creation of nanoparticles.

To ensure consistent nanoparticle nucleation and development, the reaction mixture was constantly agitated. Chemically produced Al2O3 nanoparticles were obtained by centrifuging the resultant suspension and collecting and drying the precipitate.

Figure 3: In-House chemically synthesized Al2O3 NPs

2.2 Nanoparticle Characterization

Physicochemical characterization of aluminium oxide nanoparticles (Al_2O_3 NPs) is essential to understand their structure–function relationship and to predict their biological interactions and potential toxicity. In the present study, the synthesized nanoparticles were comprehensively evaluated using a combination of spectroscopic, microscopic, and elemental analysis techniques to obtain detailed insights into their optical, structural, morphological, and compositional properties.

UV–Visible spectroscopy was employed to investigate the optical characteristics of the nanoparticles. This technique is based on the absorption of ultraviolet or visible light by electrons, resulting in electronic transitions, primarily from the valence band to the conduction band. The presence of a characteristic absorption peak is indicative of nanoparticle formation, while analysis of the absorption edge enables estimation of the optical band gap. This information is critical for understanding nanoparticle reactivity and their potential to induce oxidative stress in biological systems.

X-ray Diffraction (XRD) analysis was carried out to determine the crystalline nature and phase composition of the synthesized nanoparticles. The technique operates on Bragg's Law, wherein incident X-rays are diffracted by atomic planes within the crystal lattice. The resulting diffraction pattern provides information on crystal

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structure, phase purity, and crystallite size. Crystallinity is an important parameter influencing nanoparticle stability, surface reactivity, and interaction with biological environments.

Fourier Transform Infrared Spectroscopy (FTIR) was used to identify the functional groups and chemical bonding present on the nanoparticle surface. FTIR is based on the absorption of infrared radiation by molecular bonds, leading to vibrational transitions characteristic of specific functional groups. The identification of surface functional groups is particularly important, as surface chemistry governs nanoparticle interactions with biomolecules, cellular membranes, and proteins.

Transmission Electron Microscopy (TEM) was utilized to examine the morphology, size distribution, and aggregation state of the nanoparticles. TEM employs a high-energy electron beam transmitted through an ultra-thin specimen to generate high-resolution images based on electron scattering. This technique provides direct visualization of nanoparticle shape and size at the nanoscale, which are key determinants of cellular uptake, biodistribution, and toxicity.

Energy Dispersive X-ray Spectroscopy (EDX), typically coupled with electron microscopy, was used to confirm the elemental composition of the nanoparticles. When the sample is bombarded with high-energy electrons, it emits characteristic X-rays specific to each element. The resulting spectra allow for qualitative and semi-quantitative identification of elements, ensuring the presence of aluminium and oxygen and confirming the absence of unwanted impurities.

Inductively Coupled Plasma–Atomic Emission Spectroscopy (ICP–AES) was employed for precise quantitative analysis of elemental composition. In this technique, the sample is ionized in a high-temperature plasma, and the emitted light at characteristic wavelengths is measured. The intensity of the emitted radiation is directly proportional to the concentration of the element. ICP–AES is highly sensitive and plays a critical role in determining aluminium content and detecting trace contaminants, which is essential for accurate toxicological interpretation.

Collectively, these techniques provide complementary insights into the physicochemical characteristics of Al_2O_3 nanoparticles. Such comprehensive characterization is crucial for confirming successful nanoparticle synthesis, understanding structure–toxicity relationships, ensuring reproducibility and standardization, and accurately interpreting developmental and neurobehavioral outcomes observed in biological studies.

2.3 Zebrafish Maintenance and Embryo Collection

Adult zebrafish (Danio rerio) were maintained under standardized laboratory conditions in accordance with established zebrafish husbandry protocols [14,18]. Fish were housed in a recirculating aquaculture system at a controlled temperature of 28 ± 1°C, with a 14:10 h light–dark photoperiod, ensuring optimal physiological and reproductive performance. Water quality parameters, including pH, dissolved oxygen, and conductivity, were routinely monitored and maintained within recommended ranges[14].

The fish were fed twice daily with a combination of live feed (Artemia nauplii) and commercially available formulated diets to ensure balanced nutrition.

For breeding, sexually mature males and females were selected and placed in breeding tanks in a 2:1 ratio (male:female) during the evening prior to spawning, separated by a divider. Spawning was induced the following morning upon light exposure [18]. Fertilized embryos were collected within 4 hours post-fertilization (hpf) to ensure developmental synchrony.

Collected embryos were rinsed and maintained in E3 embryo medium under controlled laboratory conditions. Only viable and normally developing embryos were selected for subsequent experiments [13].

2.4 Exposure Paradigms

2.4.1 Fish Embryo Toxicity (FET) Assay

Developmental toxicity of aluminum oxide nanoparticles (Al_2O_3 NPs) was assessed using the Fish Embryo Toxicity (FET) assay, performed in accordance with the Organisation for Economic Co-operation and Development Guideline 236 -OECD, 2013[19].

Embryos at 4 hpf were exposed to graded concentrations of Al_2O_3 nanoparticles in sterile multi-well plates under controlled laboratory conditions. Each treatment group included appropriate controls and replicates. The

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exposure medium was renewed every 24 hours to maintain exposure consistency and minimize metabolite accumulation.

Embryos were observed until 120 hpf, encompassing key stages of embryogenesis and early larval development [19]

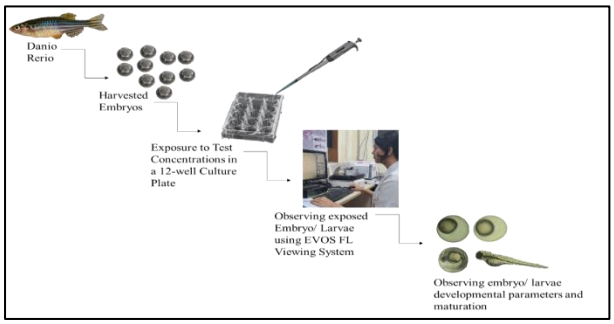

Figure 4: Flow of fish embryo toxicity setup

Endpoints Assessed: *The following endpoints were evaluated as per OECD recommendations:*

- **Survival rate** – determined by embryo viability and coagulation
- **Hatching rate** – percentage of successfully hatched embryos
- **Morphological abnormalities**, including:
    - Pericardial and yolk sac edema
    - Body curvature and spinal deformities
    - Notochord defects
    - Delayed or abnormal development

Observations were recorded at 24-hour intervals using a stereomicroscope. Representative micrographs were captured to support morphological evaluations (OECD, 2013; [20]

2.4.2 Behavioural Analysis of Larvae

Neurobehavioral assessment was conducted at **5 days post-fertilization (dpf)** to evaluate potential neurotoxic effects of aluminum oxide nanoparticles, a developmental stage at which zebrafish larvae exhibit stable and quantifiable locomotor behaviour [21].

Larvae were individually transferred into 24-well plates containing fresh E3 medium (one larva per well) and acclimatized for **20 minutes** prior to recording to minimize handling stress.

Behavioural activity was recorded using the ZebraBox **(**ViewPoint Behavior Technology**)** automated video tracking system. The system was operated under controlled environmental conditions to ensure reproducibility.

Recording Conditions:

- **Total recording duration:** 30 minutes per session
- **Light–dark paradigm:**
    - 10 minutes light phase (baseline activity)
    - 10 minutes dark phase (stimulated locomotor response)
    - 10 minutes light phase (recovery/adaptation)
- **Illumination intensity:**
    - Light phase: ~500–600 lux
    - Dark phase: <10 lux
- **Frame rate (acquisition):** 25 frames per second (fps)
- **Temperature during recording:** maintained at **28 ± 1°C**

Tracking and Analysis Parameters

Behavioural data were acquired and processed using ZebraBox software with the following standardized settings:

- Detection threshold: optimized grayscale contrast to distinguish larvae from background
- Minimum movement threshold: 0.2–0.3 mm to exclude background noise
- Immobility threshold: <0.2 mm movement
- Burst (darting) threshold: >20 mm/s instantaneous velocity
- Tracking mode**:** centroid-based tracking

Behavioural Parameters Assessed

- Swimming speed (mm/s): indicator of neuromuscular coordination

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- Total distance travelled (mm): measure of locomotor activity
- Zone preference: time spent in central vs peripheral regions (thigmotaxis behaviour)
- Darting be**haviour:** frequency of high-velocity burst movements associated with stress response

Behavioural responses were analysed across light and dark phases to assess **stimulus-dependent locomotor changes**. Quantitative data were used to determine **dose-dependent neurobehavioral alterations**, which were further correlated with developmental toxicity endpoints for an integrated toxicological assessment [21,22].

2.4.3 Chronic Exposure Studies On Adult Zebrafish and Behavioural Assessment

Adult zebrafish were acclimatized and exposed to Al_2O_3 nanoparticles at concentrations of 6, 9, and 11 µg/mL, along with a control group, for 7, 14, and 21 days, representing acute, sub-acute, and chronic exposure periods, respectively. These concentrations were selected based on the Fish Embryo Toxicity (FET) assay and estimated LC_{50} values. Exposure was carried out via water, allowing direct uptake of nanoparticles.

Each experimental group consisted of a consistent number of fish across all exposure durations.

Groups	Concentration (µg/mL)	Acute study (7 days)	Sub-acute study (14 days)	Chronic study (21 days)
1	Control	2	2	2
2	6	2	2	2
3	9	2	2	2
4	11	2	2	2
Total fishes		8	8	8

Table 1: The experimental design

2.4.4Behavioural Assessment

To evaluate neurotoxic effects, fish were individually placed in a controlled testing arena, and locomotor activity was recorded using a digital camera. Behavioural parameters including **total** distance travelled, swimming speed, and activity **patterns** were analysed using automated tracking software, providing objective insights into neurobehavioral alterations.

Novel Tank Test (NTT)

The Novel Tank Test was employed to assess anxiety-like behaviour and exploratory activity. Behavioural recordings were conducted on days 1, 7, 14, and 21. Fish were allowed to acclimatize for **1** minute, followed by a 10-minute recording period**.**

The following parameters were analysed:

- Vertical distribution (top vs bottom dwell time)
- Total distance travelled
- Swimming activity and velocity
- Freezing and erratic movements

Tracking and Data Analysis

Videos were processed using automated tracking software (idtracker.ai) and analysed using Python-based tools to generate trajectory plots, velocity profiles, and spatial distribution patterns, enabling quantitative assessment of behavioural responses.

3. Results

3.1 Physicochemical Characterization of Aluminium Oxide Nanoparticles

A comprehensive physicochemical characterization of aluminium oxide nanoparticles (Al_2O_3 NPs) was performed to understand their structural and functional properties and to predict their potential biological interactions and toxicity. The synthesized nanoparticles were analysed using a combination of spectroscopic, microscopic, and elemental techniques, and the corresponding results are presented in Figures X–X.

UV–Visible spectroscopy confirmed the successful formation of Al_2O_3 nanoparticles, as evidenced by the appearance of a characteristic absorption peak at approximately 230 nm (Figure X). The absorption profile remained consistent over time, indicating good dispersion stability of the nanoparticles in solution. Furthermore, analysis of the optical band gap using Tauc's plot (Figure X) revealed values consistent with nanoscale aluminium oxide, reflecting altered electronic properties due to quantum confinement effects. These

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optical characteristics are important in determining nanoparticle reactivity and their potential to induce oxidative stress in biological systems.

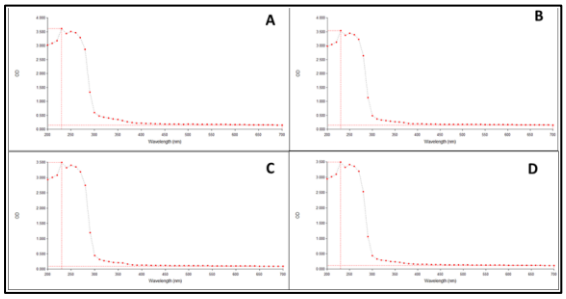

Figure 5: A-week one, B-week two, C-week three, and D-week four of the UV-Vis spectrum exhibit absorbance maxima at 360 nm, indicating the presence of aluminum oxide and its stability over a period of one month.

X-ray Diffraction (XRD) analysis demonstrated the crystalline nature of the chemically synthesized Al_2O_3 nanoparticles (Figure X). The diffraction peaks corresponded to characteristic alumina phases, confirming successful nanoparticle formation with high crystallinity. The average crystallite size was calculated using the Debye–Scherrer equation and was found to be within the nanometer range. The observed crystallinity suggests enhanced structural stability and increased surface reactivity, factors that are known to influence nanoparticle interaction with biological systems and subsequent toxicity.

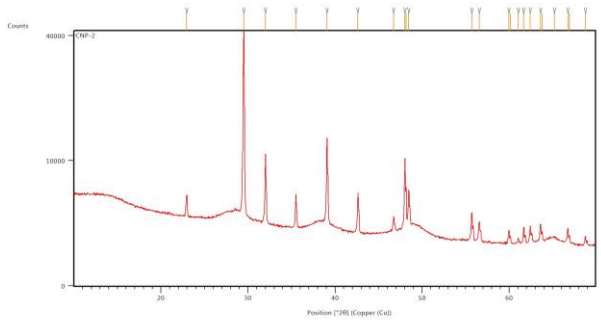

Figure 6: The XRD pattern of chemically synthesized NPs exhibited a predominantly crystalline *αα*-Alumina, which is consistent with the standard pattern for *αα*-Alumina (Alphaalumina), a white, puffy particle that is a phase of aluminum oxide, also known as alumina.

Fourier Transform Infrared Spectroscopy (FTIR) analysis further confirmed the formation of aluminum oxide nanoparticles by identifying characteristic functional groups (Figure X). Prominent absorption bands corresponding to O–H stretching, C–H vibrations, and Al–O bonds were observed, indicating the formation of Al_2O_3 nanoparticles along with the presence of residual surface functional groups. These surface moieties are critical in influencing nanoparticle behaviour in biological systems, particularly with respect to protein adsorption, membrane interactions, and cellular uptake.

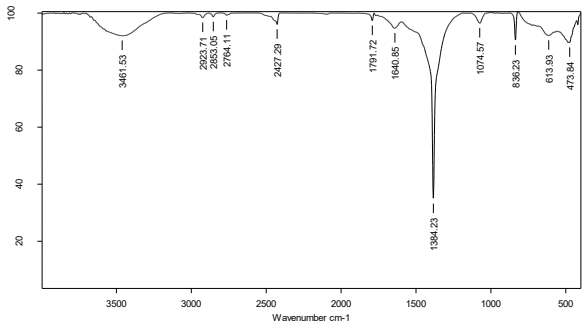

Figure 7: The FTIR spectrum of Al_2O_3 nanoparticles shows characteristic peaks corresponding to O–H stretching (~3467 cm^{-1}), C–H stretching (~2923 cm^{-1}), and carbonyl/adsorbed water vibrations (1707–1639 cm^{-1}). A distinct absorption band at ~562 cm^{-1} is attributed to Al–O lattice vibrations, confirming the formation of aluminum oxide. The presence of additional bands suggests residual surface functional groups associated with precursor molecules or adsorbed species.

Transmission Electron Microscopy (TEM) provided direct visualization of nanoparticle morphology, size, and aggregation patterns (Figure X). The synthesized Al_2O_3 nanoparticles predominantly exhibited an elongated, rod-like morphology with primary particle sizes in the nanoscale range (<20 nm). However, noticeable aggregation into clustered structures was also observed, which is typical for metal oxide nanoparticles due to high surface energy.

The observed nanoscale dimensions and anisotropic morphology are critical factors influencing cellular

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internalization, biodistribution, and overall biological interactions, thereby making TEM characterization essential for interpreting nanotoxicological outcomes.

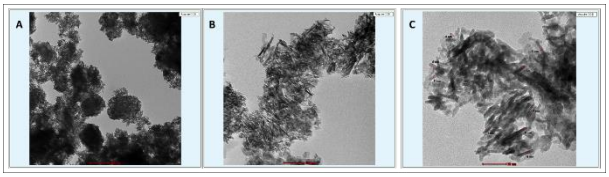

Figure 8: TEM micrographs of chemically synthesized Al_2O_3 nanoparticles showing aggregated clusters of elongated, rod-like structures. Individual particle sizes were observed in the range of ~4–8 nm, indicating fine crystalline domains. The images also reveal a tendency toward agglomeration, likely due to interparticle interactions and high surface energy.

Inductively Coupled Plasma–Atomic Emission Spectroscopy (ICP-AES) analysis confirmed the presence of aluminium in the sample, with a quantified concentration of 6.83%, reflecting its relative abundance in the prepared sample.

Collectively, these characterization results confirm the successful synthesis of chemically derived Al_2O_3 nanoparticles with defined optical, structural, morphological, and chemical properties. The integration of these complementary techniques provides a robust foundation for understanding the relationship between nanoparticle characteristics and their observed developmental and neurobehavioral effects in zebrafish larvae. Moreover, such detailed characterization ensures reproducibility, supports standardization, and enables accurate interpretation of toxicity outcomes in subsequent biological analyses.

3.2 Developmental Toxicity

Morphological assessment of zebrafish embryos exposed to Al_2O_3 nanoparticles

Exposure to chemically synthesized Al_2O_3 nanoparticles resulted in clear dose-dependent developmental toxicity in zebrafish embryos. Representative stereomicroscopic observations across developmental stages (4, 24, 48, 72, 96, and 120 hpf) revealed that embryos exposed to lower concentrations (≤4 µg/mL) exhibited normal development, including proper body elongation, somite formation, and successful hatching.

At 6 µg/mL, embryos showed early signs of toxicity, including delayed hatching and physiological stress. Increasing the concentration to 8 µg/mL resulted in significant lethality, indicating an approximate LC_{50} at this level. At higher concentrations (≥9 µg/mL), embryos displayed severe and progressive morphological abnormalities.

The observed defects included pericardial edema, yolk sac edema, curved body axis, notochord defects, eye deformities, and tail malformations, reflecting impaired embryonic development. At the highest concentrations (≥10 µg/mL), toxicity was markedly pronounced, leading to growth retardation, severe structural deformities, and increased mortality, as evidenced by the absence of viable larvae.

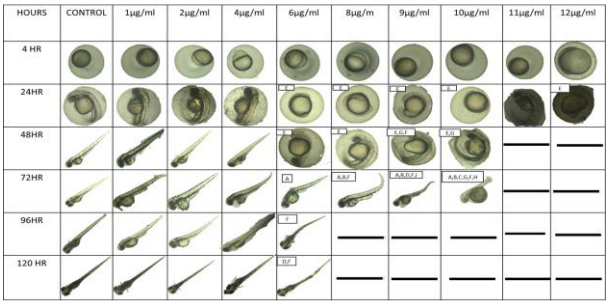

Figure 9: Representative images of zebrafish embryos/larvae at 4–120 hpf exposed to increasing concentrations of Al_2O_3 nanoparticles (0–12 µg/mL). Normal development was observed at ≤4 µg/mL, while higher concentrations (≥6 µg/mL) induced dose-dependent abnormalities including edema, body curvature, developmental delay, and reduced hatching. Severe toxicity and mortality were evident at ≥10 µg/mL. Labeled annotations indicate specific morphological defects observed during development. A – Body curvature, B – Yolk sac edema, C – Pericardial edema, D – Delayed hatching, E – Coagulated embryo, F – Tail malformation, G – Spinal deformity, H – Developmental delay

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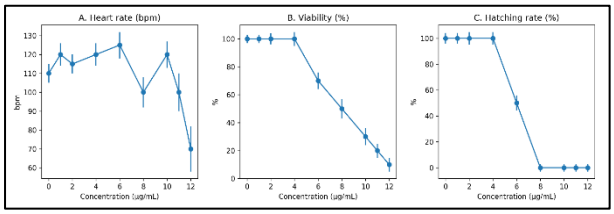


Figure 10: Dose-dependent developmental toxicity of Al_2O_3 nanoparticles in zebrafish embryos. (A) Heart rate (bpm), (B) viability (%), and (C) hatching rate (%) across increasing nanoparticle concentrations. Data are presented as mean ± SD. A concentration-dependent decline in survival and developmental parameters is observed, with pronounced toxicity at higher concentrations, particularly at ≥6 µg/mL, where significant reductions in viability, hatching rate, and alterations in cardiac activity become evident.

These findings suggest that Al_2O_3 nanoparticles may disrupt multiple developmental pathways, including neural, cardiac, and skeletal systems.

3.3 Behavioural Alterations

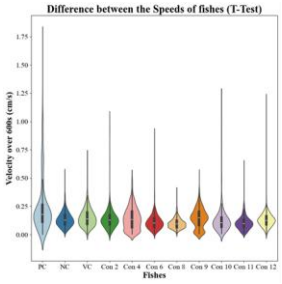
3.3.1 - Behavioural alterations in Larvae zebrafish

Exposure of zebrafish larvae to chemically synthesized Al_2O_3 nanoparticles resulted in a progressive, concentration-dependent suppression of locomotor activity. At lower concentrations (≤4 µg/mL), larvae exhibited normal swimming behaviour with consistent movement patterns comparable to controls. However, increasing nanoparticle concentrations led to marked behavioural impairments, including reduced swimming speed, restricted movement trajectories, decreased exploratory activity, and increased periods of inactivity.

Velocity distribution analysis further supported these observations, revealing a significant reduction in swimming activity in treated groups compared to controls. Control larvae displayed higher and more uniform velocity profiles, indicative of normal neuromuscular function. In contrast, exposed larvae showed lower median velocities with increased variability, suggesting impaired coordination and altered behavioural responses.

At higher concentrations, larvae exhibited minimal movement with confined trajectories and reduced velocity, along with occasional irregular bursts of activity followed by hypoactivity. These patterns indicate disrupted locomotor function and potential neurodevelopmental impairment.

Collectively, these findings demonstrate that Al_2O_3 nanoparticles induce dose-dependent neurobehavioral toxicity during early developmental stages, likely mediated through mechanisms such as oxidative stress and disruption of neural signaling pathways. These behavioural alterations are consistent with and complement the observed morphological and developmental toxicity.



Fish	Average Speed (cm/s)
Vehicle Control (VC)	0.165182
Pos Control (PC)	0.248761
Neg Control (NG)	0.1357
Con 2	0.14241
Con 4	0.139024
Con 6	0.112921
Con 8	0.100807
Con 9	0.14638
Con 10	0.12941
Con 11	0.10377
Con 12	0.132686

Figure 11 : Velocity distribution of zebrafish larvae following exposure to varying concentrations of Al_2O_3 nanoparticles. Violin plots represent the distribution of swimming speeds (cm/s) across control and treated groups. A concentration-dependent reduction in locomotor activity is observed, with exposed groups showing decreased median velocity and increased variability compared to controls, indicating neurobehavioral alterations.

3.3.2 Behavioural alterations in Adult zebrafish

Kernel Density Estimation (KDE) and locomotor analyses revealed pronounced dose- and time-dependent alterations in spatial behaviour of adult zebrafish following chronic exposure to chemically synthesized Al_2O_3 nanoparticles.

Control fish exhibited uniform exploration of the tank, reflecting normal locomotor activity and low anxiety-like behaviour. In contrast, exposed groups showed progressive behavioural disruptions with increasing concentration. At 6 µg/mL, fish displayed mild alterations, including increased peripheral preference and reduced central exploration, indicative of early stress responses.

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At 9 µg/mL, behavioural changes became more evident, with fish exhibiting restricted movement patterns and localization within specific zones, suggesting altered exploratory behaviour and emerging anxiety-like responses. At the highest concentration (11 µg/mL), severe behavioural impairment was observed, characterized by marked confinement to limited regions, reduced overall exploration, and irregular spatial distribution, indicative of impaired locomotion and neurotoxicity.

Temporal analysis demonstrated that these effects intensified with prolonged exposure (Day 1 to Day 21). Early stages showed mild confinement, particularly at higher concentrations, while Day 7 was marked by increased thigmotaxis and peripheral behaviour. By Day 14, fish exhibited significant spatial restriction and reduced exploratory activity. By Day 21, severe impairment was evident, with near-complete movement restriction at higher concentrations (9–11 µg/mL), reflecting cumulative neurotoxic effects.

In addition to spatial changes, exposed fish showed reduced locomotor activity, increased bottom-dwelling, and enhanced thigmotaxis, all of which are indicative of anxiety-like behaviour. Similar patterns of hypoactivity and abnormal swimming behaviour have been reported as hallmarks of nanoparticle-induced neurotoxicity [23].

Notably, behavioural responses followed a non-monotonic dose-response pattern. At 6 µg/mL, fish exhibited mild anxiety-like behaviour with reduced exploration. At 9 µg/mL, a transient phase of increased activity and exploration was observed, possibly reflecting neurostimulatory effects. However, at 11 µg/mL, fish displayed an initial hyperactive response followed by progressive hypoactivity and movement restriction, indicating chronic neurotoxicity and physiological exhaustion.

Overall, KDE-based spatial mapping and locomotor analysis demonstrate that Al_2O_3 nanoparticles induce significant neurobehavioral disruption, characterized by reduced exploration, increased thigmotaxis, and progressive locomotor impairment in adult zebrafish.

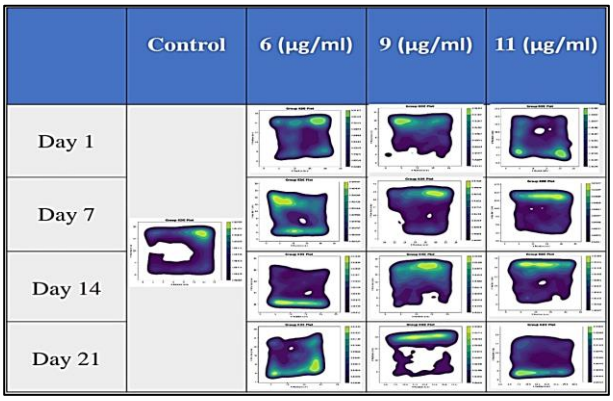

Figure 12: Kernel Density Estimation (KDE) plots showing spatial distribution of adult zebrafish under chronic exposure to Al_2O_3 nanoparticles (Control, 6, 9, and 11 µg/mL) across different time points (Day 1, 7, 14, and 21). Warmer colors indicate higher occupancy. Control fish exhibit uniform exploration, whereas treated groups show concentration- and time-dependent confinement to specific regions, reduced central activity, and increased peripheral preference, indicating altered locomotion and anxiety-like behaviour.

The observed increase in thigmotaxis, reduced exploration, and spatial restriction suggests heightened anxiety and impaired cognitive function. These behavioural changes may be associated with chronic stress responses mediated via activation of the hypothalamic–pituitary–interrenal (HPI) axis, analogous to the HPA axis in mammals.

Furthermore, the behavioural alterations may be linked to oxidative stress and disruption of neurotransmitter systems, as proposed in previous studies [7[. Although molecular endpoints were not directly assessed, the consistent behavioural patterns observed strongly indicate underlying neurochemical and neuromotor dysfunction.

Collectively, these findings demonstrate that chemically synthesized Al_2O_3 nanoparticles induce multi-level neurobehavioral toxicity in zebrafish, affecting both early developmental stages and adult behaviour. The combination of reduced locomotion, anxiety-like responses, and altered spatial behaviour highlights the potential of Al_2O_3 nanoparticles to disrupt neural function and behavioural regulation in aquatic organisms.

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4. Discussion

This study provides comprehensive evidence that chemically synthesized aluminum oxide (Al_2O_3) nanoparticles induce significant dose- and time-dependent developmental and neurobehavioral toxicity in zebrafish (Danio rerio). The integration of physicochemical characterization, embryonic toxicity, and behavioural assays enabled a multi-level evaluation of nanoparticle-induced effects.

Physicochemical characterization (UV–Vis, FTIR, TEM) confirmed the successful synthesis of nanoscale Al_2O_3 with characteristic optical properties, functional groups, and rod-like morphology. These nanoscale features, including high surface area and aggregation tendency, are known to influence biological interactions and enhance reactivity, thereby contributing to toxicity.

The Fish Embryo Toxicity (FET) assay demonstrated that embryos exposed to lower concentrations (≤4 µg/mL) developed normally, whereas concentrations ≥6 µg/mL induced significant developmental abnormalities, including delayed hatching, pericardial edema, tail curvature, and reduced survival. These findings are consistent with previous reports on metal oxide nanoparticle-induced teratogenicity [10; 11,12]. A progressive decline in LC_{50} values over time further confirmed the cumulative nature of toxicity, aligning with findings reported by Bar-Ilan et al. (2013)[16].

Cardiac assessment revealed alterations in heart rate, including bradycardia at higher concentrations, indicating physiological stress and cardiotoxicity. Similar cardiovascular impairments have been widely reported in nanoparticle exposure studies and are considered sensitive indicators of developmental toxicity.

Behavioural analysis of larvae revealed a dose-dependent suppression of locomotor activity, characterized by reduced swimming speed, restricted movement, and increased inactivity. These findings suggest early neurodevelopmental disruption, potentially mediated through oxidative stress and neuronal dysfunction. The observed reduction in velocity and altered movement patterns are consistent with impaired neuromuscular coordination.

In adult zebrafish, chronic exposure resulted in pronounced neurobehavioral alterations, including reduced locomotor activity, increased bottom-dwelling, and enhanced thigmotaxis, indicative of anxiety-like behaviour. These findings are in agreement with previous studies reporting hypoactivity and abnormal swimming as markers of nanoparticle-induced neurotoxicity [23,10].

KDE-based spatial analysis further demonstrated progressive restriction of movement and reduced exploratory behaviour, particularly at higher concentrations (9–11 µg/mL) over 21 days. Temporal progression indicated increasing severity of behavioural impairment, suggesting cumulative neurotoxic effects.

Notably, behavioural responses followed a non-monotonic dose-response pattern. At 6 µg/mL, zebrafish exhibited mild anxiety-like behaviour, while at 9 µg/mL, transient hyperactivity was observed, possibly due to neurostimulatory effects involving dopaminergic or glutamatergic pathways [24]. At 11 µg/mL, an initial hyperactive response was followed by progressive hypoactivity, indicating chronic stress, neurofatigue, or neurodegeneration, consistent with findings reported by Chen et al. (2020)[25].

The observed behavioural and developmental alterations may be attributed to oxidative stress and disruption of neurotransmitter systems, as proposed by Boran and Şaffak (2020)[7]. Although molecular endpoints were not directly assessed in this study, the consistent behavioural patterns strongly suggest underlying neurochemical disturbances. Activation of the hypothalamic–pituitary–interrenal (HPI) axis may also contribute to the observed anxiety-like responses and chronic stress effects.

Overall, this study identifies ~6 µg/mL as a critical toxicity threshold for Al_2O_3 nanoparticles and demonstrates both acute developmental toxicity and long-term neurobehavioral impairment

5. Conclusion

This study demonstrates that chronic exposure to Al_2O_3 nanoparticles induces significant dose-dependent developmental and neurobehavioral toxicity in zebrafish. Embryonic exposure resulted in delayed hatching, pericardial edema, morphological deformities, altered cardiac activity, and reduced survival, while chronic exposure in adults led to pronounced behavioural impairments, including reduced locomotion, altered

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swimming patterns, increased thigmotaxis, and anxiety-like responses.

A non-monotonic behavioural response was observed, characterized by mild anxiety at lower concentrations, transient hyperactivity at intermediate levels, and progressive hypoactivity at higher concentrations, indicating complex neurotoxic dynamics and potential neurodegenerative processes.

Importantly, these findings carry significant environmental and human health implications. The observed toxicity at relatively low concentrations (~6 µg/mL) suggests potential ecological risks, particularly in aquatic systems where nanoparticle release from industrial, biomedical, and consumer sources may lead to bioaccumulation and adverse effects on aquatic organisms. Given the conserved biological pathways between zebrafish and higher vertebrates, these results also raise concerns regarding potential neurodevelopmental and systemic impacts in humans following chronic exposure to engineered nanoparticles.

From a future perspective, there is a critical need to integrate molecular and multi-omics approaches, including transcriptomics, proteomics, and metabolomics, to unravel the underlying mechanisms of nanoparticle-induced toxicity at a systems level. Such approaches would enable identification of key biomarkers, disrupted pathways, and early signatures of neurotoxicity, providing deeper mechanistic insights beyond phenotypic observations.

Additionally, the findings underscore the importance of developing safer-by-design nanomaterials, where physicochemical properties such as size, surface charge, coating, and aggregation behaviour are optimized to minimize biological toxicity while retaining functional utility. Incorporating green synthesis strategies and surface functionalization techniques may further reduce adverse interactions with biological systems.

Overall, this study reinforces the value of zebrafish as a sensitive and translational vertebrate model for nanoparticle toxicity assessment and highlights the need for integrated risk assessment frameworks, regulatory guidelines, and interdisciplinary research to ensure the safe and sustainable use of nanomaterials.

6. Abbreviations

Al_2O_3 NPs - Aluminium oxide nanoparticles

XRD - X-ray diffraction

TEM - transmission electron microscopy

FTIR - Fourier-transform infrared spectroscopy

FET - Fish Embryo Toxicity

NaOH - sodium hydroxide

TDS - Total dissolved solids

FET - Fish Embryo Toxicity

ROS - Reactive Oxygen Species

$Al(NO_3)_3 \cdot 9H_2O$ - Aluminum Nitrate Monohydrate

NaOH - Sodium hydroxide

NTT - Novel Tank Test

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8. Author Contributions

ST: Methodology, Investigation, Data Curation, Formal Analysis, Visualization, Writing – Original Draft.

SA: Methodology, Investigation, Validation, Formal Analysis, Writing – Review & Editing.

MT:Conceptualization, Supervision, Project Administration, Writing – Review & Editing.

AT:Investigation, Resources, Data Curation, Visualization.

SB: Investigation, Resources, Visualization, Writing – Review & Editing.

HG:Conceptualization, Supervision, Methodology, Formal Analysis, Writing – Original Draft, Writing – Review & Editing, Corresponding Author.

9. Conflict of interest:

The authors declare no conflict of interest financial or otherwise.

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References

1. Prince M, Wimo A, Guerchet M, Ali GC, Wu YT, Prina M. World Alzheimer Report 2015. London: Alzheimer's Disease International; 2015.
2. Selkoe DJ. Alzheimer's disease is a synaptic failure. Science. 2002;298(5594):789–791.
3. World Health Organization. Global status report on the public health response to dementia. Geneva: WHO; 2021.
4. Exley C. Human exposure to aluminium. Environ Sci Process Impacts. 2013;15(10):1807–1816.
5. Krewski D, Yokel RA, Nieboer E, Borchelt D, Cohen J, Harry J, et al. Human health risk assessment for aluminium. J Toxicol Environ Health B Crit Rev. 2007;10(Suppl 1):1–269.
6. Bondy SC. Low levels of aluminum can lead to behavioral and morphological changes associated with Alzheimer's disease. J Alzheimers Dis. 2016;52(2):419–426.
7. Boran H, Şaffak S. Effects of nanoparticles on brain and behaviour: Role of oxidative stress and neurotransmitter imbalance. Environ Sci Pollut Res Int. 2020;27:1–15.
8. Khan I, Saeed K, Khan I. Nanoparticles: Properties, applications and toxicities. Arab J Chem. 2019;12(7):908–931.
9. Oberdörster G, Oberdörster E, Oberdörster J. Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect. 2005;113(7):823–839.
10. Bai W, Tang T. Nanoparticle-induced developmental toxicity in zebrafish. J Appl Toxicol. 2020;40(5):643–655.
11. Hansjosten I, Rapp J, Reiner L, et al. Toxicity of metal oxide nanoparticles in zebrafish embryos. Nanotoxicology. 2022;16(3):345–360.
12. Chakraborty C, Sharma AR, Sharma G, et al. Zebrafish: A complete animal model for in vivo drug discovery and development. Curr Drug Metab. 2016;17(8):792–806.
13. Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF. Stages of embryonic development of the zebrafish. Dev Dyn. 1995;203(3):253–310.
14. Lawrence C. The husbandry of zebrafish (Danio rerio): A review. Aquaculture. 2007;269(1–4):1–20.
15. Stewart AM, Ullmann JFP, Norton WHJ, et al. Molecular psychiatry of zebrafish. Mol Psychiatry. 2015;20(1):2–17.
16. Bar-Ilan O, Albrecht RM, Fako VE, Furgeson DY. Toxicity assessments of multisized gold and silver nanoparticles in zebrafish embryos. Small. 2013;5(16):1897–1910.
17. Stengl V, Bakardjieva S, Murafa N. Preparation and photocatalytic activity of nanocrystalline Al_2O_3 prepared by precipitation methods. Mater Chem Phys. 2009;114(1):217–221.
18. Westerfield M. The Zebrafish Book: A Guide for the Laboratory Use of Zebrafish (Danio rerio). 4th ed. Eugene: University of Oregon Press; 2000.
19. OECD. Fish Embryo Acute Toxicity (FET) Test. OECD Guidelines for the Testing of Chemicals, Section 2, Test No. 236. Paris: OECD; 2013.
20. Scholz S, Fischer S, Gündel U, Küster E, Luckenbach T, Voelker D. The zebrafish embryo model in environmental risk assessment. Environ Sci Pollut Res Int. 2008;15(5):394–404.
21. Kalueff AV, Stewart AM, Gerlai R. Zebrafish as an emerging model for studying complex brain disorders. Trends Pharmacol Sci. 2013;34(2):63–75.
22. MacPhail RC, Brooks J, Hunter DL, Padnos B, Irons TD, Padilla S. Locomotion in larval zebrafish: Influence of time of day, lighting and ethanol. Neurotoxicology. 2009;30(1):52–58.
23. Yeşilbudak B. Neurotoxic effects of nanoparticles in zebrafish. 2023.
24. Gerlai R. Behavioral neuroscience of zebrafish. Prog Neuropsychopharmacol Biol Psychiatry. 2000.
25. Chen TH, et al. Neurotoxicity progression of nanoparticles in zebrafish. 2020.