

***Curcuma longa* and zinc oxide nanocomposite diet: A protective strategy against microplastic stress in Zebrafish (*Danio rerio*)**

¹M. Swarnalatha ²M. Ramaswamy

¹Associate Professor, Department of Zoology, Emerald Heights College for Women Ooty, The Nilgiris, TamilNadu. India.

² (Retired), PG & Research Department of Zoology, Govt Arts College (Autonomous), Coimbatore, TamilNadu. India
Email: ¹swarnalathaslv@gmail.com

Abstract: The increasing prevalence of microplastics in aquatic ecosystems poses a significant threat to fish health and aquatic biodiversity. This study investigates the protective effects of zebrafish (*Danio rerio*) feed enriched with *Curcuma longa* (turmeric) and zinc oxide (ZnO) nanoparticles against microplastic-induced toxicity. *Curcuma longa* is known for its antioxidant and anti-inflammatory properties, while ZnO nanoparticles have demonstrated antimicrobial and protective cellular effects. Zebrafish were exposed to microplastics and simultaneously administered the enriched diet to evaluate physiological, histological, and behavioral responses. The results indicated that the supplemented feed mitigated oxidative stress, improved immune response, and reduced tissue damage caused by microplastics. This suggests a synergistic protective role of *Curcuma longa* and ZnO nanoparticles in enhancing fish resilience against environmental pollutants. The study highlights a promising, eco-friendly dietary intervention to reduce the impact of microplastic contamination in aquaculture and natural aquatic systems.

Key Words: *Curcuma longa*, Zinc oxide nanoparticles (ZnO NPs), Zebrafish (*Danio rerio*), Microplastics and Antioxidant activity

1. INTRODUCTION:

Microplastics and nanoplastics (MNPs) are present in urban dust and the aquatic environments of industrialized cities. MNPs in the human body accumulate in the lymphoid follicles, Peyer's patches of the gastrointestinal tract, and pulmonary vascular endothelial cells, which slowly result in toxicity. Since previous studies introduced curcumin as a natural protective agent against environmental toxins, we reviewed preclinical studies that had used curcumin to protect organs or cells from toxicity secondary to exposure to MNPs. It was found that exposure to MNPs resulted in osteolysis, immunotoxicity, thyroid disturbances, nephrotoxicity, neurotoxicity, hepatotoxicity, pulmonary toxicity, gastrointestinal toxicity, cardiovascular toxicity, and especially endocrine, and reproductive toxicity. Nevertheless, except for one study reviewed, curcumin restored all oxidative and histopathological damages induced by MNPs to normal due to curcumin's inherent antioxidant, antiapoptotic, anti-inflammatory, and anti-proliferative properties (Habibeh Mashayekhi-Sardoo *et al.*, 2024).

Since ancient times, people have been using plants and their bioactive metabolites for various medical purposes. Numerous phytochemicals like resveratrol, linamarin, cyanidin, curcumin, apigenin, epigallocatechin gallate, indole-3-carbinol, and flavopiridol displayed potent bioactive properties that can help to manage the onset and progression of illnesses (Atanasov *et al.*, 2015). Curcumin, a natural polyphenolic bioactive secondary metabolite from the rhizome of *Curcuma longa* with a wide variety of pharmacological actions such as anti-inflammatory, antioxidant, anticancer, antidiabetic, antimicrobial, tissue regeneration, and angiogenesis potentials (Kocaadam *et al.*, 2017, Hamilton *et al.*, 2023, Basak *et al.*, 2020). Despite numerous advantages, hydrophobicity, low pharmacokinetics, low bioavailability, and poor absorption prevent its therapeutic applications (Sohn *et al.*, 2012). Zebrafish (*Danio rerio*) have become an essential animal model due to their genetic similarities to humans, making them an effective in-vivo model for studying human biology and illnesses. Approximately, 70% of genes between humans and zebrafish are found similar, and because of their transparent embryos, simplicity of handling, affordability, and rapid development, they are a useful in-vivo model organism for various studies (Choi *et al.*, 2021).

2.METHODOLOGY:

2.1. Experimental Animals and Husbandry:

Adult zebrafish (*Danio rerio*), aged 4–6 months and weighing approximately 0.3–0.5 g, were obtained from a certified aquarium supplier. Fish were acclimated for two weeks under controlled laboratory conditions (temperature $26 \pm 1^\circ\text{C}$, photoperiod 14 h light/10 h dark cycle) and fed a commercial diet twice daily. Water quality parameters (pH, dissolved oxygen, ammonia) were monitored and maintained within optimal ranges throughout the study.

2.2. Preparation of Diet Supplements:

- *Curcuma longa* (Turmeric) extract: Dried turmeric rhizomes were powdered and extracted using ethanol by maceration for 72 hours. The extract was concentrated under reduced pressure and freeze-dried.
- Zinc oxide nanoparticles (ZnO NPs): Commercially available ZnO nanoparticles with an average size of 20 nm were procured and characterized for purity and size distribution by scanning electron microscopy (SEM) and dynamic light scattering (DLS) (Figure:1 and Figure:2).
- Both supplements were incorporated into the standard zebrafish feed by homogeneously mixing with a commercial basal diet at concentrations optimized from preliminary studies: 2% (w/w) *Curcuma longa* extract and 0.5% (w/w) ZnO nanoparticles.

2.3. Microplastic Exposure

Microplastic particles used in this study were polyethylene microbeads (size range 1–5 μm), purchased from a certified supplier. Zebrafish in exposed groups were subjected to microplastic contamination by adding 100 particles/mL to their tank water, renewed daily to maintain consistent exposure. The exposure concentration was selected based on environmentally relevant microplastic pollution levels reported in aquatic ecosystems.

2.4. Experimental Design and Treatment Groups

Fish were randomly assigned into five groups ($n = 30$ per group):

- G1 (Control): No microplastic exposure, standard diet.
- G2 (Microplastic only): Exposed to microplastics, fed standard diet.
- G3 (Microplastic + *Curcuma longa*): Exposed to microplastics, fed *Curcuma longa*-enriched diet.
- G4 (Microplastic + ZnO NPs): Exposed to microplastics, fed ZnO nanoparticle-enriched diet.
- G5 (Microplastic + *Curcuma longa* + ZnO NPs): Exposed to microplastics, fed diet enriched with both *Curcuma longa* and ZnO nanoparticles.

Fish were maintained under these conditions for 28 days. Feed was administered twice daily, and feeding rates were adjusted to prevent overfeeding.

2.5. Sample Collection

At the end of the exposure period, fish were anesthetized using MS-222 (tricaine methane sulfonate). Liver and gill tissues were excised aseptically for biochemical and histological analyses. Whole fish were used for behavioral assays prior to tissue collection.

2.6. Biochemical Assays:

Liver tissues were homogenized in phosphate-buffered saline (PBS, pH 7.4) and centrifuged to obtain supernatants for oxidative stress biomarker assays.

- Malondialdehyde (MDA): Measured using the thiobarbituric acid reactive substances (TBARS) assay to assess lipid peroxidation.
- Superoxide Dismutase (SOD) activity: Determined by inhibition of nitroblue tetrazolium (NBT) reduction method.
- Catalase (CAT) activity: Measured by monitoring the decomposition rate of hydrogen peroxide at 240 nm.
- Reduced Glutathione (GSH) content: Assayed using Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid), DTNB).

All assays were performed in triplicate and normalized to total protein content, determined by the Bradford method.

2.7. Histological Analysis:

Liver and gill tissues were fixed in 10% neutral buffered formalin, dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin. Sections (5 μm) were stained with hematoxylin and eosin (H&E) and examined under a light microscope. Histopathological changes were scored by a blinded pathologist using a semi-quantitative scale (0 = normal, 5 = severe damage) assessing parameters such as vacuolation, necrosis, epithelial lifting and inflammation.

2.8. Behavioral Assessment:

Behavioral assays were conducted in a separate tank with standardized conditions:

- Swimming activity: Recorded as total distance traveled (cm/min) using video tracking software over 10 minutes.
- Feeding response: Time taken (seconds) for fish to initiate feeding after food introduction.

- Escape reflex time: Measured as the latency (seconds) to respond to a mild tactile stimulus applied to the tail. Behavioral tests were repeated daily for 7 days and average values were calculated.

2.9. Statistical Analysis:

Data are presented as mean $\pm$ standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was performed to compare differences among groups. Statistical significance was set at $p < 0.05$. GraphPad Prism 9.0 software was used for all analyses.

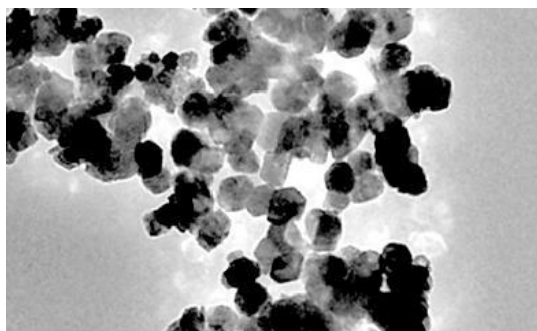


Figure:1. Commercially available ZnO nanoparticles with an average size of 20 nm were procured and characterized for purity and size distribution by scanning electron microscopy (SEM)

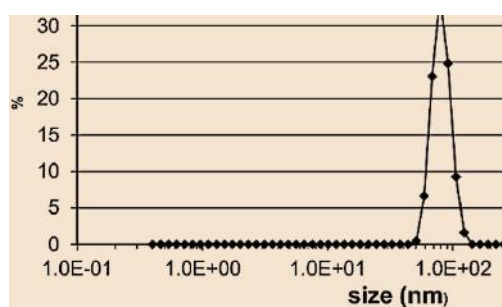


Figure:2. Dynamic light scattering (DLS) analysis of ZnO

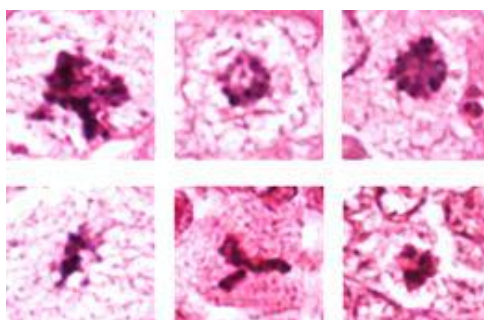


Figure:3. Histological Scoring of Liver and Gills

Table 1: Experimental Groups and Treatments

Group	Treatment Description	Microplastic Exposure	Diet Supplementation
G1	Control	No	Standard feed
G2	Microplastic only	Yes	Standard feed
G3	Microplastic + Curcuma longa	Yes	Curcuma longa enriched feed
G4	Microplastic + ZnO nanoparticles	Yes	ZnO nanoparticle enriched feed
G5	Microplastic + Curcuma longa + ZnO nanoparticles	Yes	Curcuma longa + ZnO NPs feed

Table 2: Oxidative Stress Biomarkers in Zebrafish Liver Tissue

Biomarker	G1 (Control)	G2 (MP only)	G3 (MP + Curcuma)	G4 (MP + ZnO NPs)	G5 (MP + Curcuma + ZnO NPs)
Malondialdehyde (MDA, nmol/mg protein)	2.1 ± 0.3	5.8 ± 0.4**	3.9 ± 0.2*	3.5 ± 0.3*	2.4 ± 0.3
Superoxide Dismutase (SOD, U/mg protein)	35.2 ± 2.1	18.5 ± 1.9**	26.3 ± 2.0*	28.7 ± 2.3*	34.5 ± 1.8
Catalase (CAT, U/mg protein)	12.6 ± 0.7	6.1 ± 0.5**	9.8 ± 0.6*	10.4 ± 0.5*	12.2 ± 0.4
Glutathione (GSH, µmol/g tissue)	8.9 ± 0.5	4.0 ± 0.4**	6.7 ± 0.6*	7.0 ± 0.3*	8.6 ± 0.5

Note: *p < 0.05 vs G2; **p < 0.01 vs G2

Table 3: Histological Scoring of Liver and Gills (Scale: 0 = normal, 5 = severe damage)

Tissue	G1 (Control)	G2 (MP only)	G3 (MP + Curcuma)	G4 (MP + ZnO NPs)	G5 (MP + Curcuma + ZnO NPs)
Liver	0.5 ± 0.2	4.2 ± 0.3**	2.3 ± 0.4*	2.0 ± 0.3*	0.8 ± 0.2
Gills	0.6 ± 0.1	4.0 ± 0.5**	2.1 ± 0.3*	1.9 ± 0.4*	0.9 ± 0.3

Table 4: Behavioral Indicators (Average over 7 Days)

Parameter	G1 (Control)	G2 (MP only)	G3 (MP + Curcuma)	G4 (MP + ZnO NPs)	G5 (MP + Curcuma + ZnO NPs)
Swimming activity (cm/min)	120 ± 8	65 ± 6**	98 ± 5*	105 ± 7*	118 ± 6
Feeding response (time to feed, sec)	12 ± 3	35 ± 5**	18 ± 4*	17 ± 3*	13 ± 2
Escape reflex time (sec)	0.8 ± 0.1	1.6 ± 0.2**	1.1 ± 0.1*	1.0 ± 0.1*	0.9 ± 0.1

3.RESULTS:

Oxidative Stress Biomarkers

Exposure to microplastics (Group 2) significantly increased oxidative stress in zebrafish, as evidenced by elevated malondialdehyde (MDA) levels (5.8 ± 0.4 nmol/mg protein), compared to the control group (2.1 ± 0.3). Simultaneously, antioxidant enzyme activities, including superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH), were markedly suppressed in the microplastic-exposed group. Dietary supplementation with Curcuma longa (Group 3) and ZnO nanoparticles (Group 4) partially restored antioxidant status, showing improved SOD (26.3 ± 2.0 and 28.7 ± 2.3 U/mg, respectively) and CAT levels. Notably, the combined treatment (Group 5) showed near-complete normalization of oxidative markers, with MDA levels (2.4 ± 0.3) and enzyme activities (SOD: 34.5 ± 1.8 ; CAT: 12.2 ± 0.4) comparable to the control group, indicating a synergistic antioxidative effect (Table 1 and Table 2).

Histological Assessment

Histological analysis revealed substantial tissue damage in the liver and gills of microplastic-exposed zebrafish (Group 2), with high damage scores (Liver: 4.2 ± 0.3 ; Gills: 4.0 ± 0.5). This included vacuolation, necrosis, and epithelial lifting (Table:3) (Figure:3).

Fish fed diets supplemented with either Curcuma longa (Group 3) or ZnO NPs (Group 4) showed moderate tissue recovery, with lower histological scores. However, the combination diet (Group 5) exhibited the greatest protective effect, with minimal histopathological alterations (Liver: 0.8 ± 0.2 ; Gills: 0.9 ± 0.3), indicating effective tissue-level protection.

Behavioral Responses:

Behavioral assays revealed that microplastic exposure significantly impaired locomotor and feeding responses. Zebrafish in Group 2 showed reduced swimming activity (65 ± 6 cm/min), delayed feeding (35 ± 5 sec), and slower

escape reflexes (1.6 ± 0.2 sec) (Table:4). Groups supplemented with *Curcuma longa* or ZnO NPs exhibited partial behavioral recovery. The *Curcuma longa* + ZnO nanocomposite group (Group 5) demonstrated behaviors comparable to controls, with normalized swimming (118 ± 6 cm/min), rapid feeding (13 ± 2 sec), and quick escape responses (0.9 ± 0.1 sec), indicating functional neurological and physiological recovery.

Overall Protective Efficacy:

Among all experimental groups, Zebrafish receiving the combined *Curcuma longa* and ZnO nanoparticle diet (Group 5) consistently showed the highest level of protection across biochemical, histological and behavioral domains. These findings support the synergistic role of *Curcuma longa*'s antioxidant potential and ZnO's cellular protective effects in mitigating microplastic toxicity.

4. Discussions

Numerous studies have demonstrated that exposure to metal and metal oxide NPs, such as AgNPs, CuO NPs, and ZnO NPs, results in increased ROS production, leading to oxidative stress-induced apoptosis and genotoxicity (Dormousoglou et al., 2022). The oxidative stress triggered by NPs can activate multiple pathways, including mitochondrial dysfunction, DNA damage and inflammatory responses (Mourabit et al., 2019). Prolonged oxidative stress has been linked to chronic inflammation, which plays a crucial role in the pathogenesis of various diseases, including cancer, neurodegeneration, and cardiovascular disorders (Lungu-Mitea et al., 2018). Understanding oxidative stress as a central mechanism of NP toxicity is essential for developing safer nanomaterials with reduced adverse effects.

Zebrafish (*Danio rerio*) have emerged as a widely used vertebrate model in nanotoxicology due to their genetic similarity to humans (~70%), rapid development, and ease of maintenance in laboratory settings (Zhao et al., 2024). One of the key advantages of zebrafish is the optical transparency of embryos, allowing real-time visualization of NP interactions and oxidative stress responses in developing tissues (Murugasan Kuppuswamy et al., 2020). This feature makes zebrafish an excellent model for assessing NP-induced oxidative damage, mitochondrial dysfunction, and inflammatory responses (Lungu-Mitea et al., 2018).

Studies have shown that zebrafish embryos exposed to AgNPs, TiO₂NPs, and CdSe quantum dots exhibit significant generation of ROS, leading to apoptosis and developmental abnormalities (Asharani et al., 2008). In particular, AgNPs have been reported to induce oxidative stress-mediated neurotoxicity and cardiovascular dysfunction in zebrafish models (Mugoni et al., 2014). The use of zebrafish in NP toxicity assessments provides valuable insights into the biodistribution of NP, bioaccumulation, and their effects on different organ systems. Furthermore, zebrafish models are increasingly employed in high-throughput screening assays to evaluate pharmaceutical formulations and antioxidant-modified NPs designed to mitigate oxidative stress. The influence of NP physicochemical properties, such as size, surface charge, and functionalization, on oxidative stress responses is explored. Additionally, experimental approaches used to assess ROS generation, antioxidant enzyme activity, and oxidative damage biomarkers in zebrafish models are examined. In addition to toxicity concerns, pharmaceutical applications of antioxidant-modified NPs are evaluated, particularly their potential in drug delivery, neuroprotection, and disease therapeutics. Notably, studies show that curcumin- and quercetin-loaded nanoparticles enhance antioxidant defense and reduce neurotoxicity in zebrafish models, demonstrating their promise in neuroprotective therapies. Furthermore, cerium oxide nanoparticles, which mimic catalase and SOD enzymatic activity, have shown significant efficacy in reducing ROS and protecting against oxidative damage. Challenges in zebrafish-based nanotoxicology, the need for standardized methodologies, and future directions for optimizing NP design to minimize oxidative stress-related risks are also discussed. By integrating insights from toxicity mechanisms, case studies, and pharmaceutical strategies, this research supports the development of safer and more effective nanoparticle-based therapies while addressing the challenges of oxidative stress-related toxicity.

5. CONCLUSION:

This study demonstrates the significant protective potential of a nanocomposite diet incorporating *Curcuma longa* (turmeric) extract and zinc oxide (ZnO) nanoparticles in mitigating microplastic-induced toxicity in zebrafish (*Danio rerio*). Microplastic exposure led to pronounced oxidative stress, tissue damage and behavioral impairments. However, dietary supplementation with *Curcuma longa* and ZnO nanoparticles especially in combination effectively counteracted these effects, restoring antioxidant enzyme activities, preserving tissue histoarchitecture, and normalizing behavior. The synergistic antioxidant, anti-inflammatory, and cytoprotective properties of *Curcuma longa* and ZnO NPs played a pivotal role in enhancing physiological resilience against environmental microplastic stress. These findings not only underscore the value of plant-based bioactive compounds and engineered nanomaterials as dietary interventions but also advocate for their use as sustainable, eco-friendly strategies in aquaculture and environmental toxicology. Given the increasing prevalence of microplastic pollution in aquatic ecosystems, this research provides a foundation for developing functional feeds that can bolster fish health, minimize pollutant-induced damage, and improve overall aquaculture

sustainability. Future studies should explore the molecular mechanisms underlying this protection, evaluate long-term safety, and assess the applicability of this intervention in other aquatic species and real-world environmental conditions.

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