

Exploring *Moringa oleifera* as a Natural Antidote to ZnO Nanoparticle Toxicity in Freshwater Fish

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ABSTRACT

Abstract— This study explores the potential of *Moringa oleifera* as a natural antidote to mitigate the toxicological effects of zinc oxide nanoparticles (ZnO NPs) in freshwater fish, specifically Nile tilapia (*Oreochromis niloticus*). With the increasing use of nanoparticles in various industries, their unregulated release into aquatic environments poses significant risks to aquatic life. In this experiment, tilapia was exposed to varying concentrations of ZnO NPs (50 mg/L and 85 mg/L) with or without supplementation of *M. oleifera* leaf powder for 28 days. Histopathological examinations were conducted on the liver, gills, kidney, and muscle tissues, while hematological and biochemical parameters were also analyzed. Results indicated that higher concentrations of ZnO NPs caused severe tissue damage, including necrosis, sinusoidal dilation, and lamellar disorganization. However, co-administration of *M. oleifera* demonstrated protective effects, reducing oxidative stress, preserving tissue architecture, and improving hematological indicators. These findings support the antioxidant and anti-inflammatory properties of *M. oleifera*, highlighting its potential application as a natural feed additive to counteract nanoparticle toxicity in aquaculture. This research underscores the importance of developing sustainable strategies to safeguard aquatic species from nanomaterial exposure and advocates for the integration of plant-based remedies in aquaculture practices.

Keywords: *Moringa oleifera*, Zinc oxide nanoparticles, Nile tilapia, Nanotoxicology, Histopathology

Introduction

Nanotechnology has rapidly emerged as a transformative field with extensive applications in medicine, agriculture, cosmetics, and environmental remediation (Khan *et al.*, 2024b; Aslam *et al.*, 2025a; Umar *et al.*, 2025). Among various

nanoparticles, zinc oxide nanoparticles (ZnO NPs) are widely utilized due to their antimicrobial properties, chemical stability, and low production cost (Asghar *et al.*, 2015; Sirelkhatim *et al.*, 2015). However, their increasing discharge into aquatic environments has raised serious ecological concerns. Once released, ZnO NPs interact with aquatic organisms, potentially disrupting biological processes at the cellular and systemic levels (Saxena *et al.*, 2024). Fish, being at a critical trophic level in freshwater ecosystems, are particularly susceptible to nanoparticle-induced toxicity, making them effective bioindicators of environmental stress (Khan *et al.*, 2022; Umar *et al.*, 2024a).

Exposure to ZnO NPs has been associated with oxidative stress, lipid peroxidation, and structural damage to vital organs such as the liver, gills, and kidneys in fish (Khan *et al.*, 2024a; Khan *et al.*, 2024b). These nanoparticles can generate reactive oxygen species (ROS), leading to inflammation, apoptosis, and dysfunction in tissue morphology (Khan *et al.*, 2017; Shaffique *et al.*, 2024; Aslam *et al.*, 2025b). Histopathological changes such as necrosis, pyknotic nuclei, lamellar disarray, and epithelial lifting have been widely reported in nanoparticle-exposed fish (Sayed *et al.*, 2020). In addition, hematological and biochemical parameters are significantly altered, indicating compromised physiological function and overall health deterioration (Umar *et al.*, 2024b). The increasing incidence of such effects emphasizes the urgent need for identifying strategies to mitigate the negative impact of nanomaterials on aquatic life (Saleem & Zaidi, 2020).

Natural plant-based remedies offer a promising alternative to synthetic additives for reducing nanoparticle toxicity in aquaculture (Vijayaram *et al.*, 2024). *Moringa oleifera*, known for its potent antioxidant, anti-inflammatory, and antimicrobial properties, has attracted considerable attention as a feed supplement (Mohai Ud Din *et al.*, 2025). Rich in bioactive compounds like flavonoids, phenolics, vitamins, and minerals, *M. oleifera* has demonstrated the ability to enhance immunity, reduce oxidative stress, and promote tissue repair in aquatic species (Chhikara *et al.*, 2021). Its affordability and accessibility further support its suitability for sustainable aquaculture systems, especially in developing regions. Despite its recognized potential, limited studies have investigated its protective role against metal oxide nanoparticle toxicity in freshwater fish (Mahmud & Haque, 2025).

Therefore, this study was undertaken to evaluate the protective effects of *Moringa oleifera* leaf powder against ZnO nanoparticle-induced toxicity in Nile tilapia (*Oreochromis niloticus*). The primary aim was to assess histopathological, hematological, and biochemical changes in tilapia exposed to different concentrations of ZnO NPs, both with and without *M. oleifera* supplementation. The rationale behind this study is rooted in the growing need for eco-friendly, cost-effective, and natural interventions to combat environmental nanotoxicity in aquaculture. By understanding the mitigating effects of *M. oleifera*, this research seeks to contribute to the development of sustainable fish farming practices while safeguarding aquatic biodiversity.

MATERIAL AND METHODS

Experimental fish

15 randomly chosen fingerlings of the freshwater fish Nile tilapia, weighing 20–30g

and measuring 10–15 cm in length, were bought from the Head Balloki fishpond and brought to the fisheries lab at the University of Okara's Zoology Department in Renala Khurd, Pakistan. During transit, no fatalities were reported. Every fish was examined for any harmful infections before being moved to an aquarium. For the experiment the fish were transferred to glass aquariums which were filled with tap water and allowed to acclimate to laboratory conditions for seven days. Water quality was maintained by means of a recirculating aeration system (RAS) with a water replacement system every day, removing the uneaten food and fish waste. Throughout the 28-day experiment, the following parameters were maintained: a pH of 7.46 ± 0.28 , a concentration of oxygen of 7.25 ± 0.23 mg/L, and a temperature of $24.5 \pm 2.7^\circ\text{C}$.

Experimental design

Throughout the 28-day trial, fish were kept in ponds with a 40-liter water capacity. The fish were fed commercially produced food once every 24 hours during the trial. It was preferable to put fish of comparable sizes in the same group. Two variations of *Moringa oleifera* leaf powder and zinc oxide nanoparticles were made and set up as two treatment groups. Group 3 was exposed to a combination of 50 mg/L of zinc oxide nanoparticles and Moringa leaf powder while the Group A2 was exposed to 85 mg/L of zinc oxide nanoparticles. The control (Group 1) was not exposed to any zinc oxide. Following clearance from the University of Okara's zoology department's ethical council in Renala Khurd, Pakistan, all fish handling guidelines were assumed.

Table of experimental design

NO. of Groups	ZnO NPs	MO powder	Fish Feed
1: Control group	Nil	Nil	8 grams
2: Group A1	50mg/L	2-3 grams	8 grams
3: Group A2	85mg/L	3-5 grams	8 grams

Experimental chemical

Punjab Agrochemical Trader located in Okara; Pakistan was the source for procuring zinc oxide nanoparticles. In accordance, the leaves of *Moringa oleifera* were processed and to get a standard solution of ZnO, an appropriate amount of ZnO was dissolved in distilled water.

Preparation of ZnO nanoparticles

The Department of Nanotechnology, University of Okara, Pakistan was used to synthesize zinc oxide nanoparticles (ZnO-NPs) by chemical precipitation with the assistance of ultrasonic radiation. The process started by preparing a zinc acetate dihydrate solution as it was water soluble and zinc ions are released readily upon dissolution. In order to achieve a 0.1M concentration of zinc ions, the amount of zinc acetate required was dissolved in 100 ml of distilled water. The formation of the targeted nanoparticles was the result of this solution. An aquatic ammonia solution (NH_3) with a concentration of 30 to 33% was used as the reducing agent to induce the

precipitation of ZnO. To help produce zinc hydroxide, which solidified into ZnO-NPs under controlled circumstances, a calibrated amount of the solution of ammonia was given to the zinc acetate solution while stirring to alter the pH of the mixture. An ultrasonic processing Hielscher UP400S with a 400 W power output and a 24 kHz frequency was used to ultrasonically treat the reaction mixture. In order to promote nucleation, suppress coalescence, and disperse the nanoparticles, ultrasonic treatment is essential.

During the 5-minute ultrasonic treatment process at 40 °C, the power in the ultrasound signals was kept at 79% with a duty cycle of 0.76. Because it gave the system the energy it needed to improve the reaction kinetics and architectural regularity of the encapsulating nanoparticles in the solution, ultrasonic irradiation played a crucial role in the short-term synthesis of ZnO-NPs.

After the ZnO-NPs were synthesized, the nanocomposite was filtered and cleaned to get rid of any leftover precursors and other related impurities. The finished product was then dried at a low temperature to preserve the nanoparticles' shape. When combined with the ultrasonic synthesis method, this chemical precipitation method offers several benefits, including controlled manufacturing, efficiency, and potential uses of synthesized ZnO-NPs for a range of aquaculture, biomedical, and environmental applications with desirable properties.

Preparation of Moringa Leaf Powder

Clean fresh leaves of *Moringa oleifera* should be harvested from mature plants and shouldn't be contaminated in any way by pesticides or diseases.

- Use running water that has been prepared with deionized water to wash the particles off the leaf and its surface.
- To lower the leaves' moisture content to 10–15%, place them under a lamp of warmth set to 40–50 °C for two to three days, or let them dry in the sun if you can.
- The Moringa leaves are then dried before being chopped or ground into a fine powder. As mentioned below, this will constitute the primary source of the protein, vitamins, and minerals required to prepare fish feed.

Nanoparticle exposure

The control group comprised the first group which was not exposed to zinc oxide nanoparticles. The second group (A1) were exposed to the concentration of 50 mg/L of zinc oxide nanoparticles. Comparatively, A2 group was similarly designated as the third group. It included 85 mg/L of zinc oxide nanoparticles, which were measured using a beaker.

***Moringa oleifera* exposure**

There was no exposure to *Moringa oleifera* in the control group. Nearly 5g of *Moringa oleifera* powder was given to Group A1. Three grams of moringa powder and a high amount of zinc oxide nanoparticles were administered to Group A2.

Tissue collection

Fish were gathered after being exposed for 28 days, and any remaining water was

gently removed with a dry cloth. Fish nets were used to catch fish. Using a surgical blade, the fish's belly skin was cut from the ventral side, and the liver and gill tissues were extracted and placed in preservative tubes with a 10% formalin solution. Six fish were chosen at random from each group, and their tissues were taken out for histological examination.



Figure 1: Tilapia fish in unconscious state before dissection.

Histological study

In this study, the liver and gill tissues of several Nile tilapia (*Oreochromis niloticus*) from each treatment group were removed for pathological examination following the histological method described by Spencer et al. (2012). For the immunohistochemical analysis of kidney and muscle tissues, samples were initially fixed in Bouin's fluid, consisting of glacial acetic acid, formalin, and saturated aqueous picric acid. After fixation, tissue dehydration was carried out through sequential immersion in increasing concentrations of ethanol (80%, 90%, 95%) for 15 minutes and 70% ethanol for 24 hours. The tissues were then cleared in xylene and embedded in paraffin. Samples were first treated with a paraffin-xylene mixture and baked for two hours at 60° C, followed by light paraffin embedding for another two hours, and finally hardened in paraffin for 24 hours at the same temperature. Sectioning was performed using a microtome to produce 2 – 3 mm thick slices, which were mounted on slides pre-coated with Mayer's albumin, prepared according to Humason (1962). Histological staining was performed using Hematoxylin and Eosin (H&E) staining techniques. The hematoxylin stain was prepared by dissolving 1.0 gram of hematoxylin powder in one liter of distilled water, with additional components including citric acid, potassium alum, chloral hydrate, and sodium iodate. The eosin stain was made by dissolving eosin powder in ethanol, followed by the addition of picric acid, potassium dichromate, and distilled water, with a drop of glacial acetic acid for enhancement. These procedures enabled the examination of blood and tissue samples for hematological and biochemical variations induced by different doses of ZnO nanoparticles (ZnO NPs) and *Moringa oleifera*. Observations included changes in blood cell morphology, cholesterol levels, and overall health indicators. Histopathological evaluation revealed structural anomalies in liver tissues, such as damage to glomeruli, renal tubules, and interstitial tissues, offering insight into the

toxicological and physiological impacts of the experimental treatments on fish health.

Microscopy and analysis

For histological examination, the liver and gill slides from every treatment and control group were examined under a trinocular light microscope. A camera mounted on a tripod was used to take pictures straight from the eyepiece lens.

Ethical Approval

Every experiment involving live fish was conducted in compliance with the Veterinary Animal Management and Use Standards. The study was approved by the University of Okara's Renala Khurd Zoology Department's Ethical Review Committee. To cause the experimental animals as little discomfort as possible, standard humane handling procedures were applied.

RESULTS

This is a chapter in which bloods of Nile tilapia (*Oreochromis niloticus*) with exposure to *Moringa oleifera* and ZnO NPs (ZnO NPs) were investigated. Secondly, the study aimed to find out different hematological and biochemical variations due to the administration of different quantities of ZnO NPs and Moringa A. pterygosperma in the blood cell shape, cholesterol levels, and overall health. The results were arranged to major functions of the synthesized ZnO NPs and Moringa, fish growth performance, fish body condition, water quality indication, hematological anomalies, histological changes, toxicity, cell culture assay, different lipid profile, and various statistical methods. This offer details the physical and chemical modifications to tilapia as a result of ZnO NPs and Moringa so that they can be used in the fishing industry or water filtration. This species incorporates three data chapters to establish context for additional discussion and interpretation of health effects on Nile tilapia that were a result of many of the other treatments.

The tissues responses for all the experimental groups are broken up into separate sections in this chapter part. The Histological Analysis of Liver Tissues describes the structural integrity and anomalies of the glomeruli, renal tubules, and interstitial spaces. In a similar manner, The Histological Analysis of Gills Tissues addresses fiber organization, necrotic alteration, and cellular degeneration. The Examination of Liver and Gills Tissues that are presents the integration of the main pathogenic patterns across the groupings. Finally, a visual comparison of the tissue reactions to various experimental conditions is provided in Microscopic Measurements and Staining Observations.

The results are presented methodically using supporting microscopic data, which demonstrate the degree of cell recovery that Moringa may cause and the degree of ZnO NP toxicity that can be imparted. This chapter's topic serves as a foundation for debates on the biological importance of exposure to ZnO NPs in aquatic creatures, nanotoxicity, and antioxidant intervention. The findings made it easier to comprehend the exposure conditions that lead to oxidative stress, how oxidative stress caused by nanoparticles contributes to cell death, and how different chemical species of nanoparticles interact with naturally occurring oxidative defenses (i.e., antioxidant molecules), which are thought to be prototypes for potential therapeutic uses.

Histopathology

This study has been done to investigate the histopathological changes in Nile tilapia after exposure to ZnO NPs for 28 days and has considered the liver and the gills as the organs analyzed for histological changes.

Liver tissues of control group A

Under a camera-equipped microscope, the Nile tilapia liver segment (from the control group) displayed normal histology with no changes noted. The liver cells of this group of fish show typical morphology, with foamy cytoplasm, a round or polygonal cell body, and a clear, round nucleus with a nucleolus. Here we present the histological analysis of the liver tissues in Nile tilapia fed only fish feed, that is the control group. Throughout the study there were no signs of mortality or behavioral changes in the control group. In contrast, the fish in the experimental groups showed a changed behavior, including the immobility, flinching, and tendency to hide.

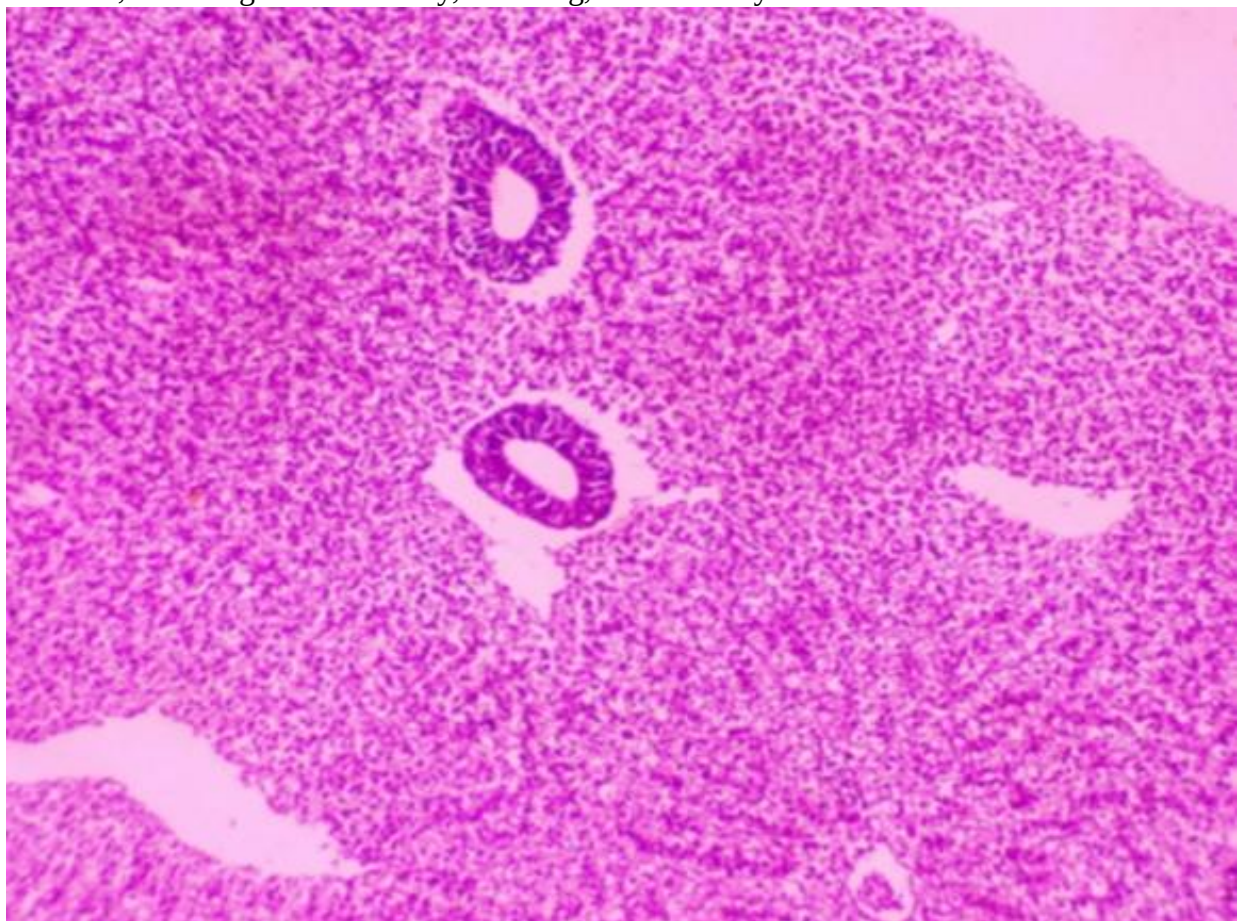


Figure 2: Normal liver cells with no alteration.

Liver tissues of group A1

Three grams of moringa powder and fifty milligrams per liter of zinc oxide nanoparticles were given to this group. The effects seen in tilapia liver tissues are as

follows. Blood congestion is indicated by a green arrow, pyknosis by a black arrow, and lymphocyte infiltration by an orange arrow. Necrosis and biological membrane disintegration are further impacts. This is a recovery group since zinc oxide nanoparticles have detrimental effects whereas moringa has beneficial effects.

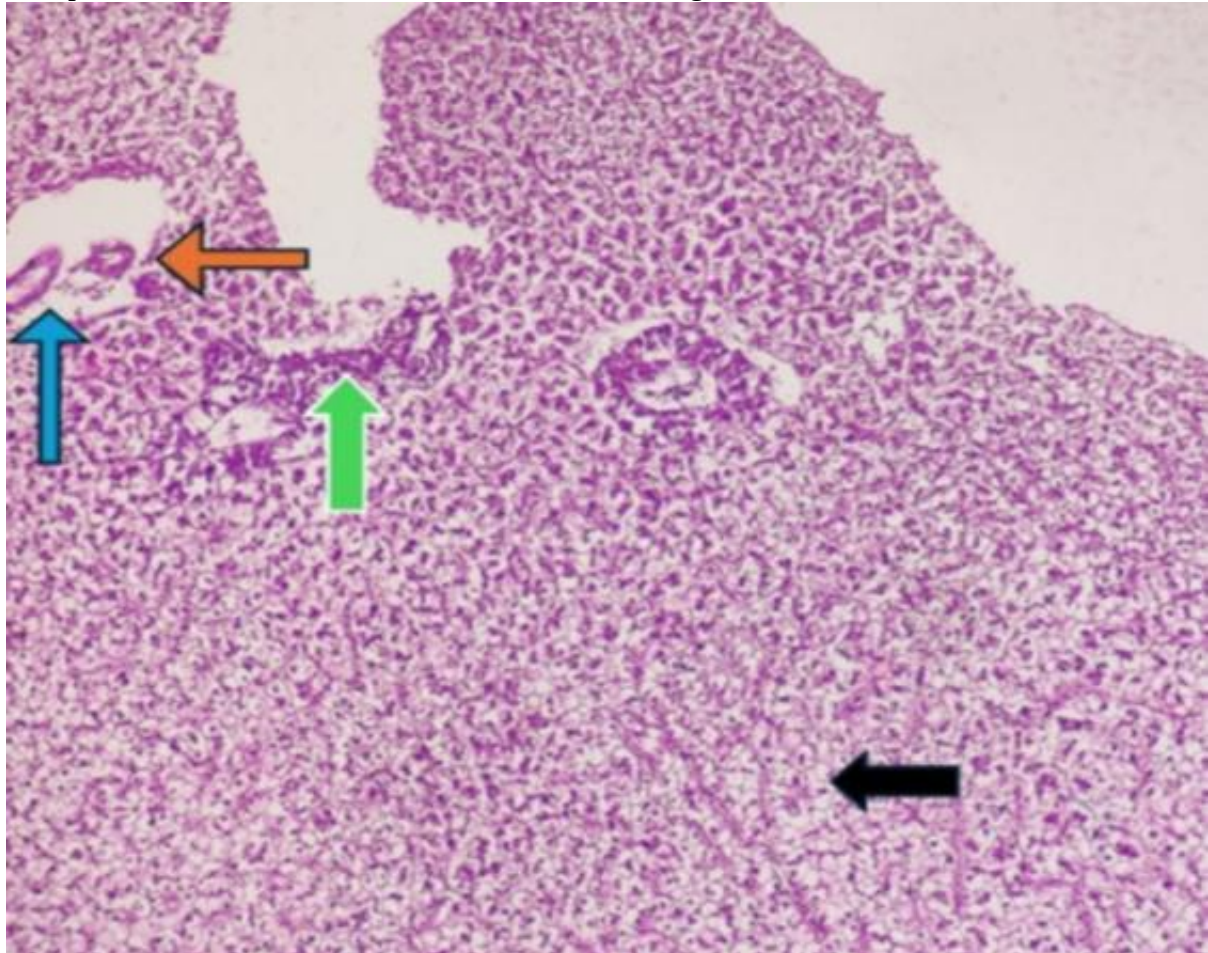


Figure 3: Histological image of effected areas by arrows of liver cells of group A1.

Liver tissues of Group A2

Five grams of powdered *moringa oleifera* and 85 mg/l of zinc oxide nanoparticles were fed to this group of species. The consequences of group A2 species' liver tissues are listed below. Pyknotic nuclei are shown by blue arrows, large sinusoidal gaps by green arrows, necrosis by red arrows, and sinusoidal dilatation by black arrows. The group that received the highest dose of ZnO NPs was the most affected.

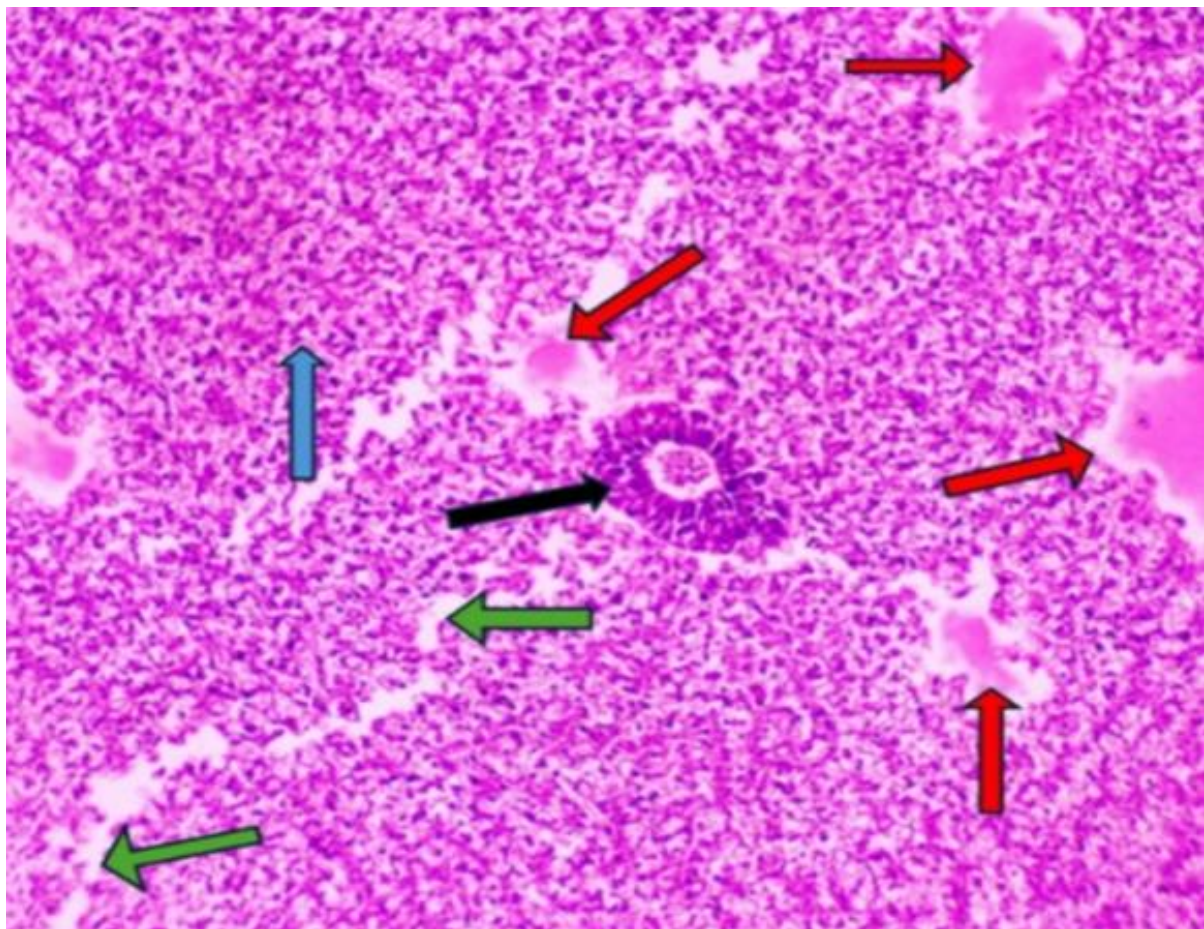


Figure 4: Histological image of effected areas by arrows of liver tissues of group A2.

Gill tissues of Control Group A

Using a camera-equipped microscope, the Nile tilapia gill section (from the control group) was examined. Its histology was found to be normal, with no changes noted in the primary and secondary gill filaments. It displays a central cartilaginous core and a typical, thin epithelial coating. mucous-lining stratified epithelium that sits between the primary and secondary gill lamellae.

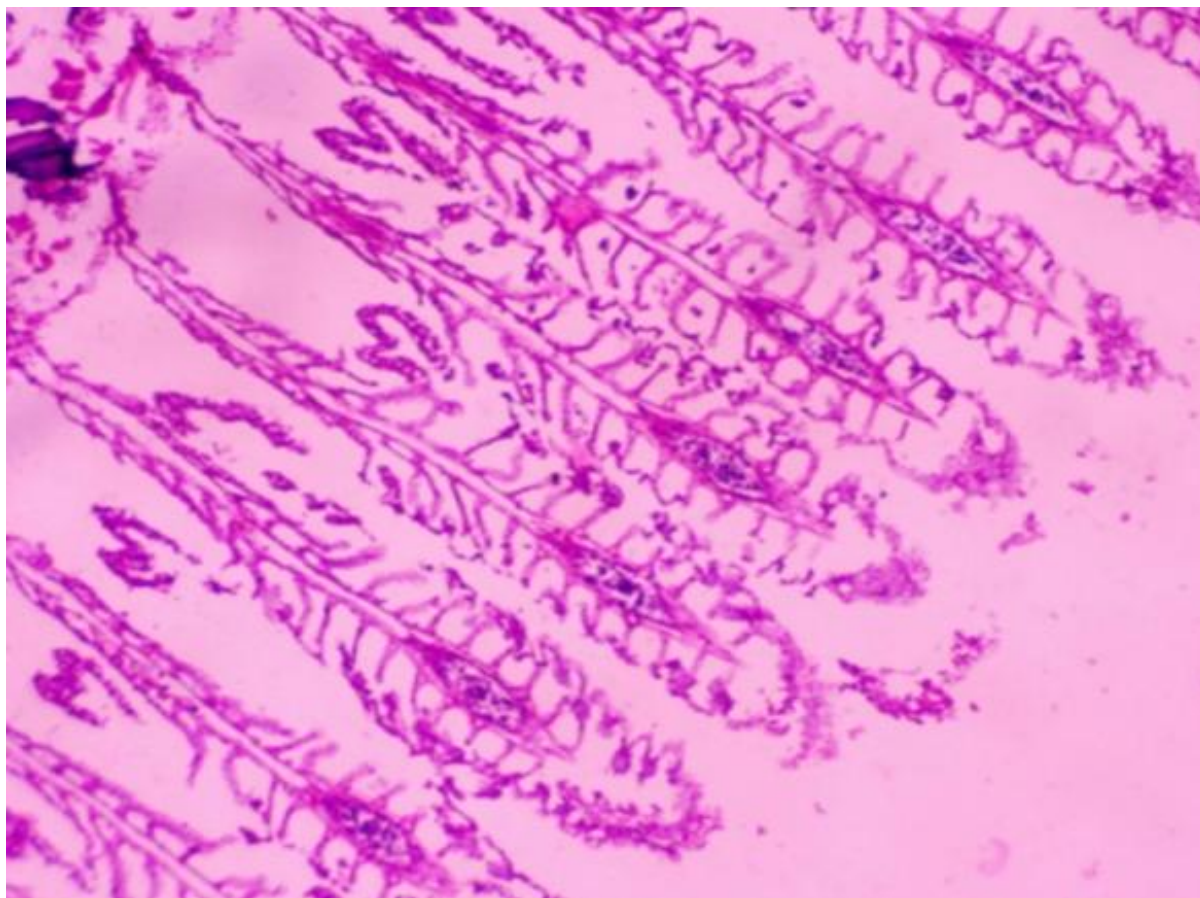


Figure 5: The histopathological examination of the gills of the control group shows appearance in form of microscopic image of normal unaltered structure of the gill anatomy.

Gills tissues of Group A1

Different arrows represent different histological abnormalities, such as lamellar aneurism (yellow arrow), bone cell malformations (red arrow), and lamellar disorganization (blue arrow). This is a group for recovery, because zinc oxide nanoparticles have both beneficial and detrimental effects on *moringa oleifera*.

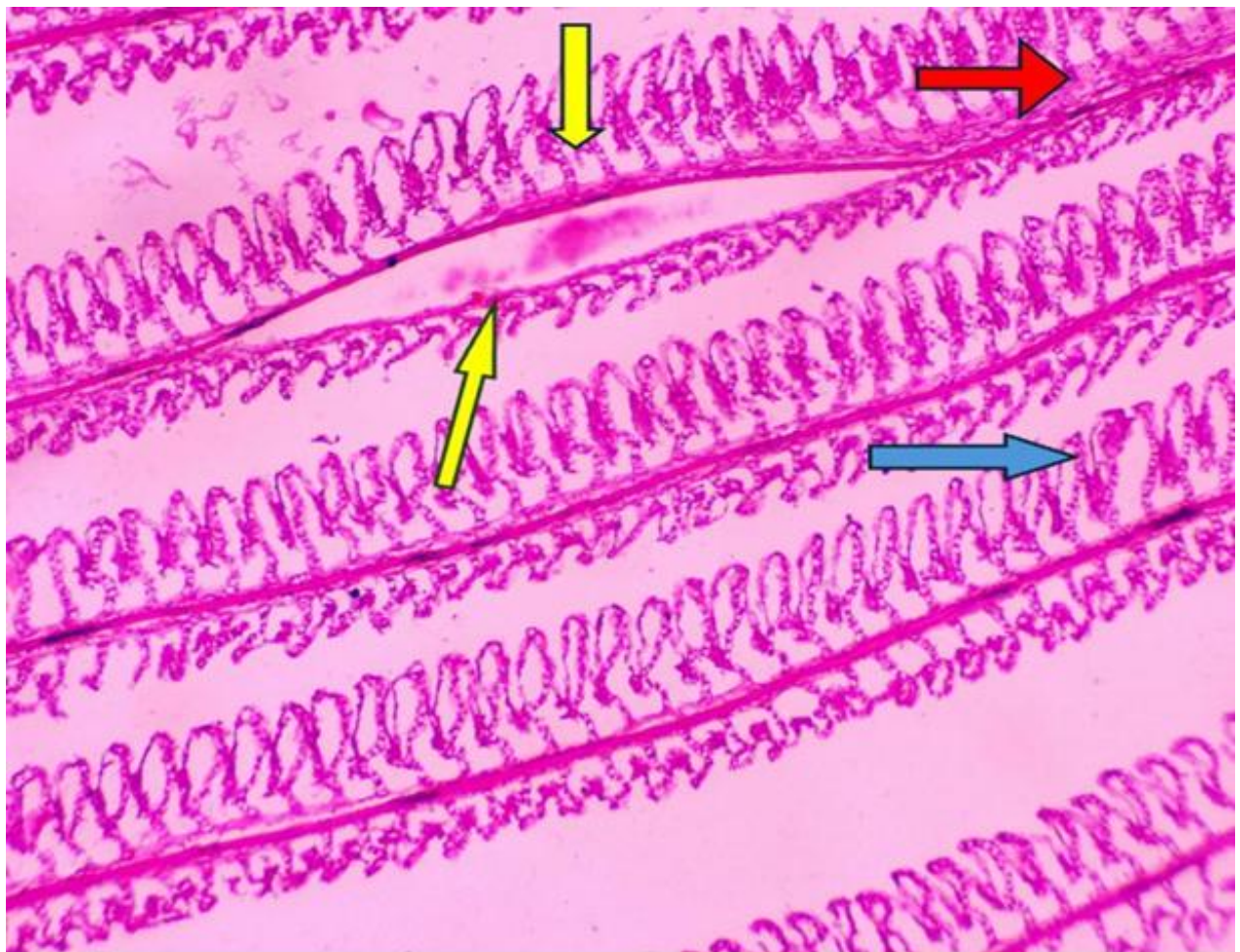


Figure 6: In this group, A1, the histopathological examination of the gills provides a microscopic image of which the affected areas of the gill tissues are designated by the arrows.

Gills tissues of Group A2

The picture displays the histological alterations following exposure to 85 mg/l of zinc oxide nanoparticles and 5 grams of moringa powder. This group is most affected. Oedema is shown by a black arrow, bone cell abnormalities by a red arrow, lamellar aneurism by a yellow arrow, bent gill lamellar by a green arrow, and lamellar disarray by a blue arrow.

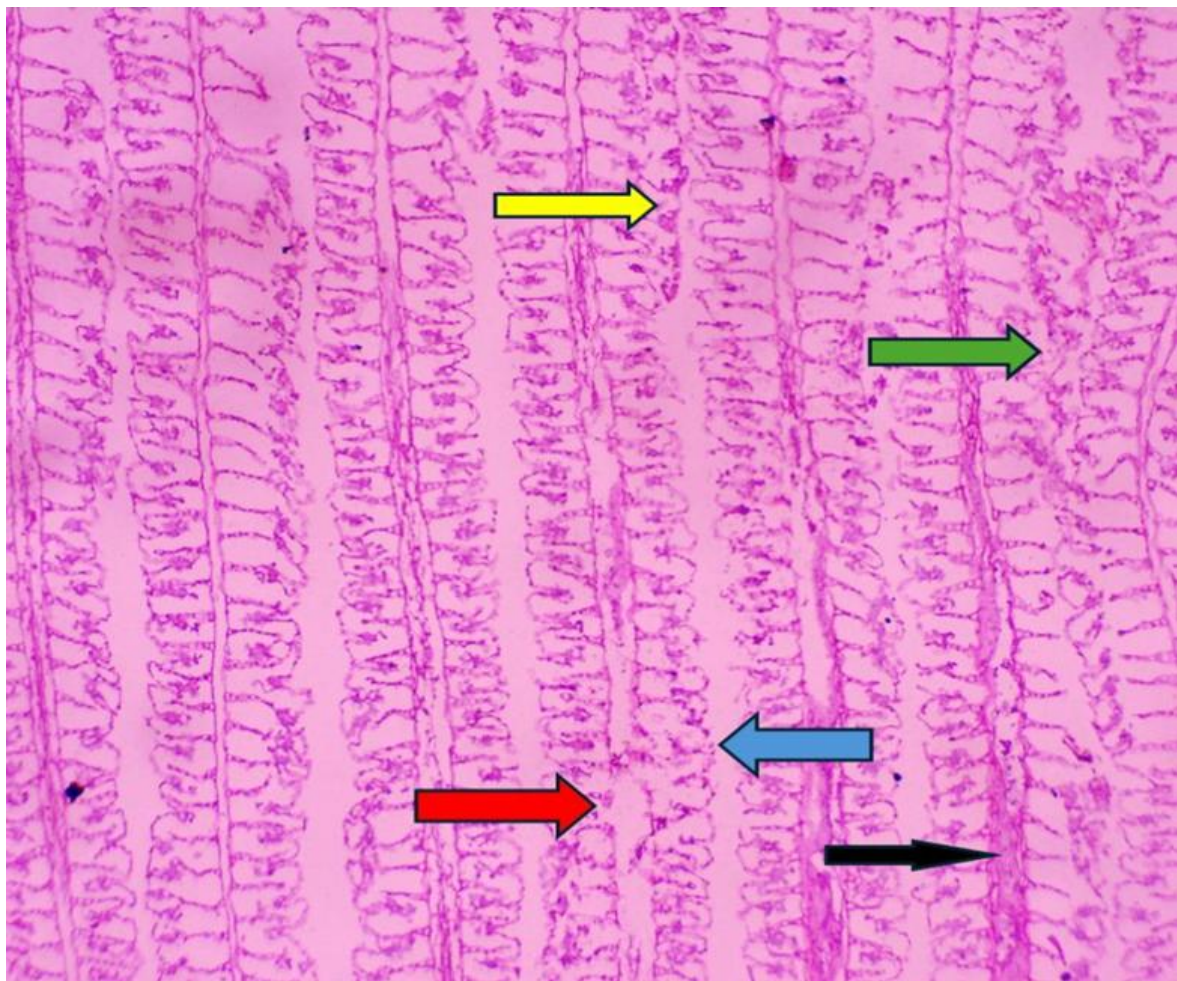


Figure 7: Histological image of effected areas by arrows of gills tissues of group A2.

DISCUSSION

The paper is of immense importance since it is able to illustrate the toxicity results of zinc oxide nanoparticle (ZnO-NP) exposure in Nile tilapia (*Oreochromis niloticus*) and the protective effect of *Moringa oleifera* leaf nanoparticles (MO-NPs) as a dietary agent. The findings of nanoparticle toxic effects of this study correlate with the other literature studies because the effects of nanoparticle exposure in literature have been widely reported to impair oxidative stress, liver, and immunosuppressive effects of aquatic organisms in literature. Liver enzyme elevation, including liver enzymes ALT and AST, reflected the similar level of no ZnO or ZnO-NP_u in the current study and indicated the measurable pronounced level of liver stress engendered and induced by ZnO-NP_s exposure, which causes the actual introduction of hepatocellular damage. The variations of the hematological indices, including the reduced hemoglobin and red blood cell tests, could also be observed, which reflected that ZnO-NPs have aggravated the integrity of the blood and the capacity to deliver oxygen. The investigation of Hamed et al. (2022) found similar biochemical distortions and made the conclusion that, having experienced the effect of ZnO-NPs in the diet, a tilapia acquires poor liver properties, a lowered ability to process lipids, and an unbalanced

immune reaction. However, the DMO-NP supplementation succeeded in alleviating these toxic effects, as the majority of the physiological parameters returned to near-normal values; therefore, *Moringa oleifera* has the therapeutic capacity to treat nanoparticle toxicity.

The toxicity of ZnO-NPs was dose dependent, whereby its severity rose with rising concentrations, resulting in biochemical and histopathological destruction. The findings are consistent with the approach conducted by Asghar et al. (2015), providing a critical assessment of the toxicity of Zn nanoparticles in fish, and a comparative toxicity study ... tilapia (2021) that have reported histopathological and immunological defects. It is also pertinent to mention that in the study in question, a ZnO-Nps concentration of 85 mg/L led to extreme manifestations of hepatic lesions (sinusoidal dilatation, nuclear pyknosis, and necrosis) as compared to 50 mg/L. These types of histological changes indicate that the ZnO-NPs can disrupt the cellular architecture along with the biochemical homeostasis. This shows a confirmation of the hypothesis that nanoparticles with a high surface area/volume ratio together with reactivity possibilities can penetrate biological membranes, causing intracellular oxidative stress, which is a known mechanism of nanotoxicity (as suggested in the general doctrines of nanotoxicology). These effects have necessitated the need to establish strict regulation powers associated with the use of nanoparticles in aquaculture and environmental systems to avoid such hazardous accumulation and ill effects, which are not only short-term but also long-term to the environment. Not only does accumulation of nanoparticles in fish affect the health and survival trends of fish, but also this exposes a potential threat to the safety of consumed foods and general health of the population since they are bioaccumulated in the food chain.

Leaves of *Moringa oleifera* nanoparticles demonstrated themselves as a highly efficient antidote to conservation of toxicity caused by ZnO-NP. In the research presented, the remarkable statistics indicate the fact that the fish that were consumed by MO-NPs depicted improved immune responses, including the improvement of the activity of lysozyme and total immunoglobulin level that points to the assertion that the immunity has been triggered. The antioxidant status also improved, and this was reflected in the normalization of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) levels and the decrease of malondialdehyde (MDA) levels, which is said to be a marker of lipid peroxidation. The conclusion is supported by El-Kassas et al. (2022), where their research has found the effects of the addition of *Moringa oleifera* leaf powder to improve the immune response to the Nile tilapia and antioxidant status. Further complementary findings are made by Mahmoud et al. (2022), which state that the presence of MO extract ameliorated fipronil-induced oxidative stress and immune suppression. The flavonoids, phenolics, vitamins, and essential minerals found in *Moringa oleifera* are components of its phytochemicals that help to scavenge free radicals and protect the cells. Additionally, structural protection was documented when the histological analysis applied in this experiment showed that tissue in the liver and gills was guarded against destruction upon the application of MO-NPs. The findings support the use of *Moringa oleifera* as a functional feed additive in order to guarantee the welfare of the fish in the face of environmental or chemical stressors.

Moreover, the beneficial effect of *Moringa oleifera* in the area of aquaculture does not

solely lie in the limits of toxicity of nanoparticles. Its ability to increase the performance of growth and feed conversion and improve immune resistance has also been tested in other toxicological situations. As an example, Abdelhiee et al. (2021) demonstrated an increased growth and feed conversion using low concentrations of dietary Moringa. Also, Kamble et al. (2024) showed heightened resistance towards *Streptococcus agalactiae*, and Nassar (2024) showed immunomodulatory and growth-inducing effects towards *Aeromonas hydrophila*. Moreover, red tilapia reproduction and performance were enhanced by the long-term supplementation procedure (Sallam et al., 2025). These findings are consistent, confirming *Moringa oleifera* as a natural bioprotective feed additive; this is inexpensive, widely available as a feed supplement, safe, and effective, particularly in areas where such synthetic supplements are limited, and it promotes resilience, water quality, and overall fish productivity.

In conclusion, it is evident that the current research indicated that nanoparticles of *Moringa oleifera* leaves have significant protective effects against the ZnO-NP-induced physiological, biochemical, and histological damage on Nile tilapia. Such findings underline the high necessity of aquaculture systems to develop and introduce the application of alternative (natural and plant-derived) interventions to minimize the use of fake additives and supersede the dangers created by nanotechnology. The aim of the study was to overview toxic effects of ZnO-NPs and assess protection potential of MO-NPs on the basis of biological systems. The justification is the growing industry in nanoparticles, where adverse release to the environment indicates the possibility of uncontrollable environmental releases. The use of the well-known, medicinally well-studied *Moringa oleifera* provides a foundation on which further analyses of dose optimization, prolonged effects, and inter-specific applicability may be performed. It reflects the necessity of the need to interdisciplinary merge nanotoxicology, pharmacognosy, and aquaculture nutrition to guarantee sustainable and harmless aquatic food production.

CONCLUSION AND RECOMMENDATION

This study aimed to investigate the reduction in the availability of the ZnO-NPs to the aquatic organisms and the impact of *Moringa oleifera* and the ZnO-NPs on the blood physiology of Nile tilapia (*Oreochromis niloticus*). The pollutants observed were found to have an adverse effect on the tilapia fish, however, *Moringa oleifera* powder was found to have beneficial effects. The accumulation of nanoparticles in fish bodies over a 28-day exposure period has been linked to negative toxicological alterations in biochemical traits and histological abnormalities in tilapia (*Oreochromis niloticus*). Following exposure to both nanoparticles, the liver and gills' normal histology changed. In this work, we demonstrate how exposure to nanoparticles damages aquatic life and changes its physical properties. All practical measures must be made to keep the levels of nanoparticles in the marine environment from developing and growing above permissible limits.

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