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THE INFLUENCE OF TITANIUM DIOXIDE (TIO2) NANOPARTICLES ON HAEMATOLOGICAL PARAMETERS OF FISH

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ABSTRACT
The utilisation of titanium dioxide (TiO2) nanoparticles (NPs) in the production of consumer goods has experienced significant growth, raising concerns about their potential to cause harmful impacts on aquatic organisms. Previous studies have shown the potential adverse effects of metal oxide nanoparticles (NPs) on aquatic creatures. However, limited information is available on the cytotoxicity and genotoxicity of titanium dioxide (TiO2) NPs specifically on the African catfish species, *Channa punctatus*. This study aimed to examine the haematotoxicity of titanium dioxide nanoparticles (TiO2 NPs) in Channa punctatus through the utilisation of the micronucleus (MN) assay and haematological analysis. The fish were subjected to varying concentrations of TiO2 nanoparticles (NPs) ranging from 200 to 800 mg L−1 for durations of 5 and 10 days. During the exposure period, there was a significant decrease in red blood cells, haematocrit, platelets, and heterophils, accompanied by an increase in mean corpuscular haemoglobin concentration and lymphocytes. This trend persisted across the 10-day exposure period. This study demonstrates that the exposure to TiO2 nanoparticles (NPs) resulted in haematological changes in the fish species *Channa punctatus*. These findings have implications for the fields of biodiversity and aquatic health management.

Keywords: Channa punctatus, blood, titanium dioxide, nanoparticle

Introduction

The field of nanotechnology has witnessed significant progress, leading to the proliferation of nanoparticles and their widespread application across various industries, including cosmetics, textiles, and electronics. Over the years, there have been notable advancements in the synthesis of nanoparticles, leading to substantial enhancements in their physical and chemical characteristics. These advancements have played a crucial role in the greater utilisation of nanoparticles. The improper disposal of nanoparticles might result in adverse environmental effects. Nanoparticles exhibit a relatively high surface area-to-mass ratio due to their diminutive dimensions. The aforementioned phenomenon facilitates the establishment of a suitable environment for chemical reactions and results in the attachment of hazardous substances onto nanoparticles. Nanoparticles has the ability to traverse the blood-brain barrier and blood-eye barrier. Various xenobiotic effects can be caused by them. According to a recent study conducted by Mohammadbakir (2016), silver nanoparticles were found to be deposited in many organs of zebrafish

embryos, including the brain, yolk, blood, and heart. This deposition resulted in significant erosion, indicating potential adverse effects.

The utilisation of nanoparticles necessitates significant consideration of their possible ecological and health ramifications. However, the existing body of literature on this subject is limited, and a comprehensive understanding of the physiological mechanisms underlying nanomaterials remains incomplete. There is currently limited knowledge regarding the potential positive or negative impacts of nanoparticles on fish, and the underlying mechanisms involved remain poorly comprehended. Various businesses, including the medical sector, sensor technologies, and water cleanup systems, release nanoparticles (NPs) into water bodies throughout the manufacturing and utilisation of their products. Consequently, the deposition of NPs in aquatic environments gives rise to significant health hazards for aquatic organisms. The deleterious impacts of NPs have been previously documented in numerous aquatic and terrestrial organisms.

Titanium dioxide nanoparticles (TiO2-NPs) are extensively employed synthetic materials found in various industrial applications, including textiles, coatings, cosmetics, personal care items, and food goods. These nanoparticles are valued for their exceptional anti-corrosion characteristics, durability, and strong photocatalytic activity. As a result of their inherent photocatalytic properties, these substances exhibit catalytic behaviour upon exposure to ultraviolet (UV) radiation, hence facilitating the degradation of pesticides, polychlorinated biphenyls (PCBs), and other pollutants present on surfaces that are subjected to sunlight and atmospheric conditions. When the dimensions of TiO2 nanoparticles decrease to less than 100 nm, they present a significantly greater risk to terrestrial and aquatic organisms in comparison to TiO2 particles bigger than 100 nm, which have been determined to be non-hazardous to both human health and the environment.

Several studies have been undertaken to evaluate the toxicological impact of micro and nano-scale titanium dioxide (Areecheewakul et al., 2020; Baranowska-Wójcik et al., 2020; Bobori et al., 2020; Tosco and Sethi, 2018). Several studies have reported the occurrence of negative impacts on human health (Ahamed et al., 2020; Brandão et al., 2020) as well as the biological environment (Chavan and Sarangdhar, 2020; Du et al., 2019) associated with these substances. The majority of the studies focused on animal models, with a specific emphasis on investigating the effects on the gastrointestinal system, as well as assessing potential pulmonary dangers and cutaneous exposure (Warheit & Donner, 2015). Limited research has been conducted about the impact of pure TiO2-NP on aquatic organisms, particularly with respect to prolonged exposure, as evidenced by studies conducted by Dong et al. (2020), .Previous research on the impact of nanoparticles on aquatic species primarily concentrated on investigating the effects of short-term exposure. Notably, these studies observed genotypic alterations, such as changes in ribosomal function .

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The results of a brief exposure experiment involving rainbow trout and titanium dioxide revealed the presence of gill pathologies and a reduction in Na+ K+ ATPase activities within the gills and intestines. The aforementioned research have demonstrated the presence of adverse effects caused by titanium dioxide nanoparticles on aquatic organisms. However, it is important to note that further experimentation with prolonged exposure is required to provide a more comprehensive understanding of the long-term impacts (Ramsden, Smith, Shaw, & Handy, 2009). A study examining the effects of prolonged exposure to TiO2 on zebrafish demonstrated growth inhibition that was dependent on both concentration and duration of treatment. A separate study demonstrated the aquatic toxicity of copper oxide (CuO) nanoparticles on carp, indicating that growth suppression occurred at elevated concentrations (100 mg/L) throughout a 30-day exposure period. A study conducted over a period of 21 days examined the impact of TiO2-NPs, in conjunction with paraquat, on carp. The investigation revealed alterations in the blood chemistry of the fish. The aforementioned research mostly focused on the waterborne impact of nanoparticles. However, there is a lack of published data about the dietary exposure of fish to TiO2-NPs, specifically in relation to growth indices. The present investigation was undertaken to assess the impact of dietary exposure to titanium dioxide nanoparticles (TiO2-NPs) on fingerlings of the freshwater fish species *Channa Punctatus. Channa punctatus* was selected due to its classification as one of the prominent carp species found in the Indian subcontinent, alongside *Catla catla* and *Cirrhinus mrigala*. Furthermore, due to its rapid development, high output, and favourable reception among consumers, this particular fish species is widely recognised as one of the most extensively cultivated in South Asia.

Methodology

Nanoparticle preparation

Stock solutions of TiO_2 nanoparticles were prepared using distilled water and subsequently dispersed using an ultrasonic bath. Subsequently, solutions were placed into exposure tanks containing 10 litres of dechlorinated water in order to generate TiO_2/L concentrations that were nominally distinct.

Exposure Assays

Fish that were less than one year old were assigned randomly to groups of 15 fish each in polystyrene test tanks with a volume of 15 L following the acclimatisation phase. The fish were subjected to varying quantities of TiO2 nanoparticles, ranging from 200 ppm to 800 ppm/L. The experimental group of fish was housed in a distinct aquarium containing uncontaminated tap water. The fish underwent a series of tests involving consistent aeration and temperature control. The experimental conditions of each tank were altered at regular intervals of forty-eight hours during the entire duration of the twenty-one-day test. Throughout the duration of the trial, the fish were provided with a daily ration of commercially-produced dry food flakes, which were also made available to them at their own discretion. The enumeration of deceased fish

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in the tanks was conducted in a continuous manner.

Sampling the tested organisms

Following exposure for 5 and 10 days, fish was gathered for sampling. One further fish group (n=5) were taken for sampling from the acclimation tank at the start of the trial. During each instance, following capture, the Fish were subjected to anaesthesia using clove powder at a concentration of 200 ppm. Blood samples were expeditiously collected from the tail blood vessel using needles that had been treated with heparin. Haematological analyses were conducted on recently collected blood samples. The blood erythrocytes and leukocytes were examined at a dilution of 1:30 by mixing heparinized blood with Giemsa stain in order to reduce its intensity. The enumeration of cells was conducted by employing a hemocytometer Neubauer beneath the illumination of a light microscope, as described by Stevens (1997). Hb mg/L levels were determined by quantifying the production of cyanmethemoglobin on the surfaces.

The computation of erythrocyte indices, including Mean Corpuscular Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC), and Mean Corpuscular Volume (MCV), was performed using the parameters of Ht, Hb, and RBC, as described by Lee et al. in 1998.

Statistical analysis

One-way ANOVA was used for statistical analysis of the results after the data have been evaluated for normality and homogeneity (using Leven's test) and, if necessary, correctly converted.

Results and Discussion

Red Blood Cell (RBC)

The impact of comparable exposure on red blood cells (RBCs) was shown to be diminished, as evidenced by the results presented in the figure 1. The data presented indicates that over a period of 5 days, exposure to doses of 200 milligrams per litre, 400 milligrams per litre, 600 milligrams per litre, and 800 milligrams per litre resulted in a drop in red blood cell (RBC) levels. Specifically, the RBC levels were seen to be 6.1±0, 5.0±0.0, 5.23±1.05, and 4.85±0.07, respectively, in an orderly manner. In a comparable manner, when exposed to doses of 200 milligrams per litre, 400 milligrams per litre, 600 milligrams per litre, and 800 milligrams per litre for a period of 10 days, the red blood cell (RBC) levels were recorded as 5.1±0.28, 4.10±0.0, 5.05±0.07, and 4.78±0.02, respectively. The mean value of red blood cell (RBC) control was 6.25±1.20 on the 5th day and 6±1.55 on the 10th day.

Figure 1: The mean ± SD of red blood cell (RBC) levels in *Channa punctatus* were detected after exposure to varying doses of nanoparticles for a duration of 5 and 10 days. The values are represented as the mean plus or minus the standard error of the mean (SEM) in the context of a One-Way Analysis of Variance (ANOVA). There is a statistically significant difference ($P < 0.05$) in the mean values among the treatment groups.

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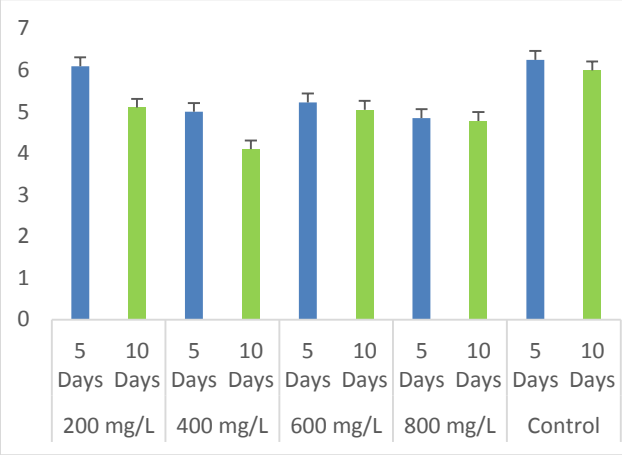

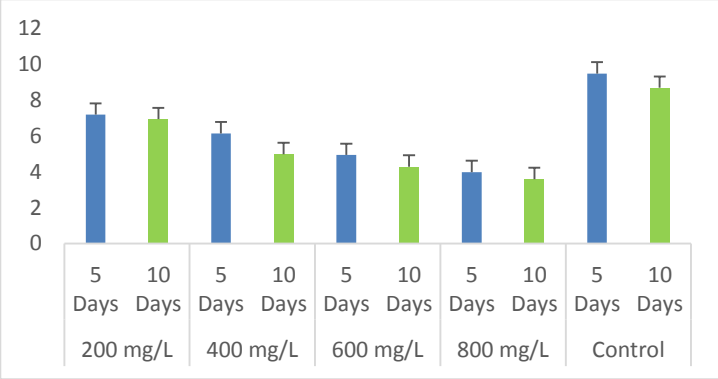

Figure 2 displays the mean ± standard deviation of haemoglobin levels in Channa punctatus after exposure to varying doses of nanoparticles for durations of 5 and 10 days. The values are shown as the mean plus or minus the standard error of the mean (One Way ANOVA). The mean values among the treatment groups exhibit statistically significant differences ($P < 0.05$), which are unlikely to have occurred by coincidence.

Haemoglobin (Hb)

Over a period of 5 days, the subjects were exposed to doses of 200 milligrams per litre, 400 milligrams per litre, 600 milligrams per litre, and 800 milligrams per litre. The levels of Hb (haemoglobin) were observed to fall by 7.2±0.42, 6.15±0.07, 4.95±0.07, and 4±0, respectively. Furthermore, when subjected to varying doses of 200 milligrams per litre, 400 milligrams per litre, 600 milligrams per litre, and 800 milligrams per litre for a period of 10 days, the haemoglobin (Hb) levels were recorded as 6.95±0.07, 5±0.14, 4.3±0.42, and 3.6±0.39, respectively. The control values of haemoglobin (Hb) were seen to be 9.5±0.70 and 8.7±0.28 on the 5th and 10th days, respectively. The impact of exposure on haemoglobin levels diminishes as the concentration increases in comparison to the control group.

Haematocrit (Hct)

Ultimately, precise dosages were administered throughout the study in order to evaluate the impact on haematocrit levels. In relation to the matter at hand, it was observed that the Haematocrit levels exhibited a drop of 15.77±0.84, 12.1±0.28, 15.70±071, and 15.11±0 throughout the five-day period of exposure to doses of 200 milligrams per litre, 400 milligrams per litre, 600 milligrams per litre, and 800 milligrams per litre, respectively. The mean corpuscular haemoglobin (MCH) levels were seen to be 15.47±1.13, 10.4±0.28, 15.28±0.54, and 14.48±0.22 following a 10-day exposure to dosages of 800 milligrams per litre, 600 milligrams per litre, 400 milligrams per litre, and 200 milligrams per litre, respectively. The control values of haematocrit (HCT)

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were measured to be 20.84±0.67 on the 5th day and 21.26±0.41 on the 10th day.

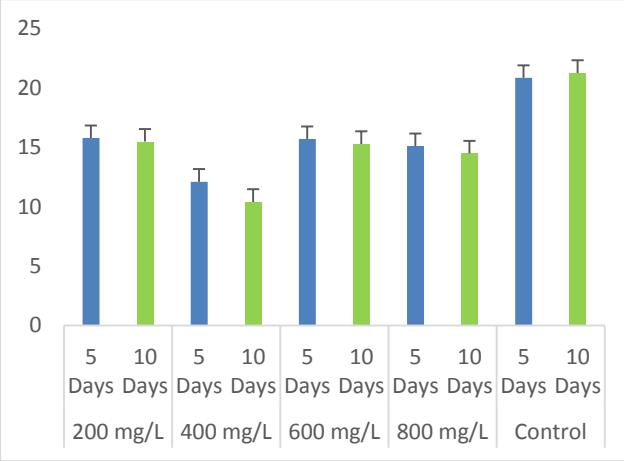

Figure 3 displays the mean±SD of haematocrit (Hct) levels in Channa punctatus, which were detected following treatment with varying doses of nanoparticles for durations of 5 and 10 days. The values are shown as the mean plus or minus the standard error of the mean (One Way ANOVA). The observed results indicate a statistically significant difference, as evidenced by a p-value of less than 0.05.

Mean cell volume (MCV):

Additionally, the experiment was conducted using identical exposure conditions to assess the effect on mean corpuscular volume (MCV) levels. The results indicated a decrease in MCV levels compared to the control group after both 5 and 10 days. Regarding the matter, it was observed that after a duration of 5 days, exposure to doses of 200 milligrams per litre, 400 milligrams per litre, 600 milligrams per litre, and 800 milligrams per litre resulted in a drop in MCV levels by 20.45±0.62, 21.80±0.84, 24.04±1.20, and 21.05±0.07, respectively. During a 10-day period, the subjects were exposed to dosages of 200 milligrams per litre, 400 milligrams per litre, 600 milligrams per litre, and 800 milligrams per litre. The resulting mean corpuscular volume (MCV) levels were seen to be 20.54±0.74, 19.6±0.59, 21.55±0.72, and 20.43±0, respectively. The mean corpuscular volume (MCV) control values were seen to be 23.0±9.87 and 20.34±5.29 for the 5th and 10th days, respectively.

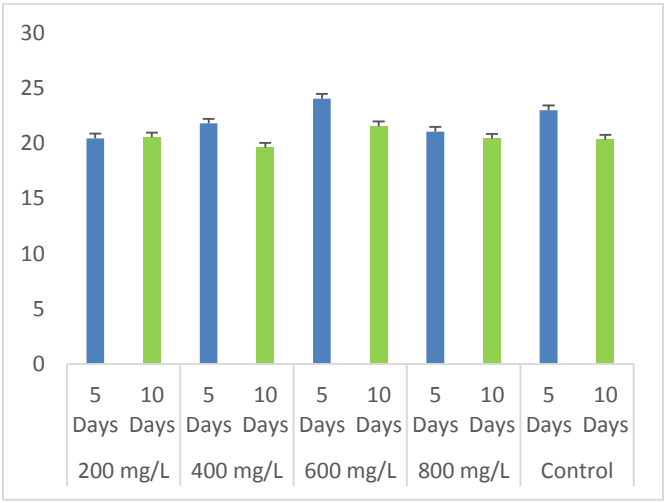

Figure 4: The MCV level (mean±SD) in *Channa punctatus* were observed on exposing to different dose of nanoparticle for 5 days and 10 days. Values are expressed as Mean±SEM (One Way ANOVA) the outcome is statistically significant i.e., ($p<0.05$).

Mean cell haemoglobin (MCH)

Furthermore, the identical degree of exposure was employed during the examination in order to ascertain the impact on mean corpuscular haemoglobin (MCH) levels. In relation to this matter, the MCH levels exhibited a decrease of 13.35±0.36, 11.35±0.07, 13.39±0.70, and 12.1±94 during the course of five consecutive days of

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exposure to doses of 200 milligrams per litre, 400 milligrams per litre, 600 milligrams per litre, and 800 milligrams per litre, respectively. During a 10-day period of exposure, the mean corpuscular haemoglobin (MCH) levels were observed to be 11.77±0.67, 10.40±0.4, 11.67±0.15, and 10.32±0.45 for dosages of 800 milligrams per litre, 600 milligrams per litre, 400 milligrams per litre, and 200 milligrams per litre, respectively. The control values for mean corpuscular haemoglobin (MCH) were 11.60±0.67 and 11.85±0.04 on the 5th and 10th days, respectively.

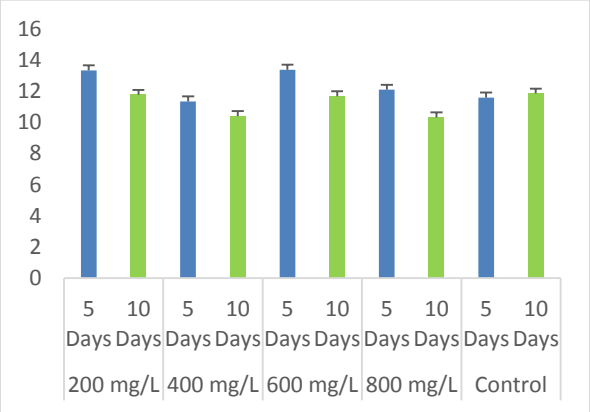

Figure 5: The MCH level (mean±SD) in *Channa punctatus* were observed at treating at different dose of nanoparticle for 5 days and 10 days. Values are expressed as Mean±SEM (One Way ANOVA). The out come is statistically significant difference ($P < 0.05$).

Mean cell haemoglobin concentration (MCHC)

Similarly, the identical intervention was employed during the evaluation to ascertain the impact on mean corpuscular haemoglobin concentration (MCHC) levels. In relation to this matter, the mean corpuscular haemoglobin concentration (MCHC) levels exhibited an increase of 24.13±0.05, 212.0±1.56, 215±0.05, and 210.27±041 during the duration of five days of exposure to doses of 200 milligrams per litre, 400 milligrams per litre, 600 milligrams per litre, and 800 milligrams per litre, respectively. During a 10-day period of exposure, the mean corpuscular haemoglobin concentration (MCHC) values were recorded as 208.62 ± 1.35, 218.36 ± 0.53, 205.77 ± 6.92, and 214.26 ± 0.40, respectively. The mean corpuscular haemoglobin concentration (MCHC) values for the 5th and 10th days were recorded as 184.35±6.33 and 182.56±8.20, respectively.

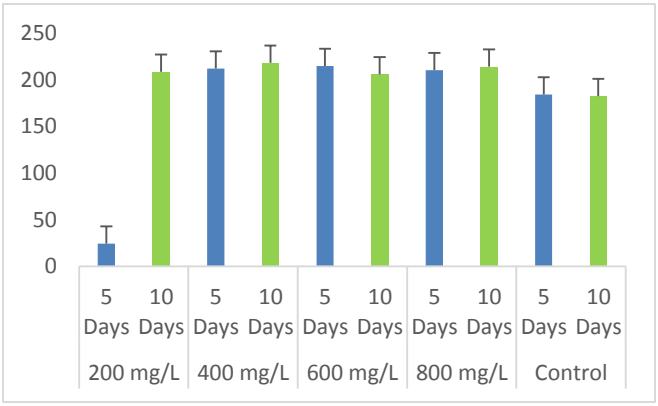

Figure 6: The MCHC level (mean±SD) in *Channa punctatus* were observed on exposingg to different dose of nanoparticle for 5 days and 10 days. Values are expressed as Mean±SEM (One Way ANOVA) the outcome is statistically significant i.e., ($p<0.05$).

White Blood Cells (WBC)

Furthermore, a consistent dosage was administered throughout the study to assess the impact on white blood cell (WBC) levels. In relation to this matter, the white blood cell (WBC) levels exhibited an increase of 23.0± 1.23, 1302.5 ±60.10, 1914 ±1.41, and 1805 ±9.89 over the course of

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five days of exposure to doses of 800 milligrams per litre, 600 milligrams per litre, 400 milligrams per litre, and 200 milligrams per litre, respectively. In contrast, the white blood cell (WBC) levels observed after a duration of 10 days were recorded as 1952.2 ±24.04, 1293.5± 7.77, 1872.5 ±7.77, and 1800.5 ±0.70, respectively. The mean values of white blood cell (WBC) count were 4133±9.19 and 4277.8±69.29 for the 5th and 10th days, respectively.

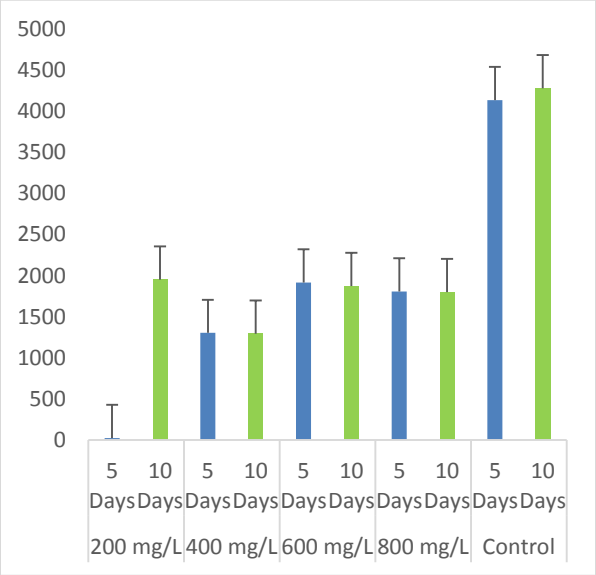

Figure 6: The WBC level (mean±SD) in *Channa punctatus* were observed on exposing to different dose of nanoparticle for 5 days and 10 days. Statically significant difference comparatively to control if ($p<0.05$).

Conclusion

The findings of this study indicate that the presence of nano titanium dioxide can have an adverse impact on blood parameters in goldfish, leading to a decrease in red blood cells and haemoglobin levels. Ultimately, this detrimental influence on fish health culminates in fish mortality.

The physiological response to nanoparticles varies among different fish species. When examining toxicity, it is important for researchers to take into account the potential negative consequences of chronic toxicity in nonmaterial substances. Based on the findings of this study, the advancement of nanotechnology and the use of its resultant products across diverse industries have the potential to inflict harm onto marine ecosystems. The findings of this investigation revealed that it was observed to have adverse impacts on the blood indices of channa punctatus, leading to a reduction in red blood cells and haemoglobin levels. Ultimately, these effects resulted in the mortality of the fish.

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