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Short Communication: Acute toxicity effects of titanium nano particle TiO_2 NPs on hematological indices in Goldfish (*Carassius auratus*, Linnaeus 1758)

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Abstract. Hedayati A, Darabitabar F, Ahmadvand S, Ahmadvand S. *2017. Short Communication: Acute toxicity effects of titanium nano particle TiO2 NPs on hematological indices in Goldfish (*Carassius auratus*, Linnaeus 1758). Nusantara Bioscience 9: 152-155.* Metal nanoparticles which include particles manufactured from metal oxides such as zinc oxide ZnO and titanium oxide TiO_2 particles are broadly used due to their inimitable attributes such as diversity level chemistries and can be prepared into a variety of figures and sizes. The aim of this study was to investigate the effect of different TiO_2 NPs concentrations on hematology indices in Goldfish (*Carassius auratus*). 105 goldfish with average total lengths of 6.50 ± 0.43 cm were used in this present study. Goldfish were exposed to a lethal concentration of 50% LC_{50} nano-titanium dioxide for a period of 0 (control), 24, 48 and 96h static toxicity examination, presented in tanks of 60L. The results of this study shows $LC50_{96h}$ nano titanium dioxide for goldfish is 19985±2.27 ppm and MAC value or the maximum allowable concentration calculated for this toxin is 1998 mg/L. No mortality observed in the control group. Fish exposed to nano-titanium dioxide for 7 days, a significant change in hematological (Hb, MCH, MCHC, MCV, RBC), and immunological (WBC) indices concentrations toward the control groups ($p<0/05$). The results of this study showed, however, LC_{50} of nano titanium dioxide was very high that means it doesn't have much mortality effect, but it has negative effect on blood indices in goldfish and reduces red blood cells and hemoglobin in fish, eventually caused the death of fish.

Keywords: Goldfish, nano titanium, blood indices, $LC50_{96h}$, metal oxides

INTRODUCTION

Metal nanoparticles which include particles manufactured from Pt, Fe, Cu, Au, Ag metal oxides such as ZnO and TiO_2 particles are broadly used due to their inimitable attributes such as diversity level chemistries and can be prepared into a variety of figures and sizes (Murphy et al. 2005). Whereas engineered nanoparticles in the nanoscale limited area of 1-100 nm have great and beneficial properties, the same can be hectic from toxicological outlook. Currently, large splits exist in our knowledge and exposure of nano materials for aquatic organisms and understanding of the toxicity, which barricade their danger evaluation. So, enhanced research is guaranteed on diversity toxicological issues related to nanoparticles. The recent upsurge in production, variety and usage of manufactured nanomaterials has raised concerns that the release of these materials into the environment may pose an earnest menace (Moore 2006; Klaine et al. 2008).

Titanium dioxide, also known as titanium (IV) oxide, naturally appeared in three forms viz., anatase, rutile and brookite. Professional exposure to TiO_2 nanoparticles can befall during their occupational exposure in the production of pigments for paints, varnishes, enamels, lacquers and paper coatings to impart whiteness, opalescence and brightness (Hext et al. 2005). The most usual oxidation state of Ti is +4, but +3 and +2 states also exist. Metallic Ti, TiO_2, and TiCl4 are the compounds most broadly used in industry. TiO_2 (CAS-No. 13463-67-7), also known as titanium (IV) oxide, titania, titanic anhydride, titanic acid anhydride, or Ti white, is the naturally befalling oxide of Ti. TiO_2 is a poorly dissolved particulate that has been broadly used as a white pigment. Anatase and rutile are two crystal structures of TiO_2, with anatase being more chemically reflexive (Warheit et al. 2007). TiO_2 is a white noncombustible and odorless powder with a molecular weight of 79.9 g/mol, pimple point of 2972°C, melting point of 1843°C, and comparative density of 4.26 g/cm3 at 25°C. Toxicity experiments may be conducted in vivo or in vitro or in silico (Bruin et al. 2015).

The classic experimental gadget of toxicology is zoological testing. Animal experiments provide data that is not accessible by other means about how materials function in a living organism (Ottoboni, 1991). Biochemical indices used in fish and other aquatic organisms in faced to heavy metal stress as the important bio- index for monitoring aquatic environment (Shalaby et al. 2005). Hematological indices are very important for the fish assessment physiological situation. The indicators include fish cycle, age, species of sexual maturity of breeders, and diseases (Golovina and Trombicky 1989).

The goldfish is a subdued version of a lower colorful

carp (*Carassius auratus*) native to East Asia. The goldfish is a freshwater fish in the family of Cyprinidae of order Cypriniformes. A relatively small member of the carp family (which also includes the crucian carp and the koi). It was first domesticated in China more than a thousand years ago, and several separate species have since been developed (Horsefall and Spiff 1998). The aim of this study was to investigate the effect of different TiO_2 NPs concentrations on hematology indices in Goldfish.

MATERIALS AND METHODS

In this study, 105 goldfish with an average total length of 6.50 ± 0.43 cm were used in this study. Acclimatized fish were fed daily with a formulated feed. Dead goldfish were immediately removed with special plastic forceps to avoid possible deterioration of the water quality (Gooley et al. 2000). Goldfish were exposed to a lethal concentration of 50% LC_{50} nano-titanium dioxide for control, 24, 48 and 96h static toxicity examination, presented in tanks of 60 L. Experiment was conducted for seven days. One group control was conserved in a fiberglass tank without accessing toxicant. For each experimental time were carried out three repeats. Dissolved oxygen, PH, water temperature and conductivity were monitored during the experiment (Hedayati et al. 2010).

At each time, upon catching, Fish were anesthetized with 200 ppm clove power. Blood samples were rapidly taken from tail blood vessel by heparinized syringes. Blood indices were carried out on fresh blood. Blood erythrocytes and leukocytes were carried out at 1: 30 dilution by attenuate heparinized blood with Giemsa stain. Cells were recounted using a hemocytometer Neubauer below the light microscope (Stevens 1997). By measuring the formation of cyanmethemoglobin were found Hb mg/L surfaces. Erythrocytes Indices (Mean Corpuscular Hemoglobin or MCH, Mean Cell Hemoglobin Concentration or MCHC and Mean Corpuscular Volume or MCV) were computed from Ht, Hb, and RBC (Lee et al. 1998).

RESULTS AND DISCUSSION

In this present study, no mortality observed in the control group. The impact of different concentrations of nano titanium dioxide at various times on goldfish is presented in Table 1. According to the results of Table 1, as well as using the software probit analysis values LC_1, LC_{10}, LC_{30}, LC_{50}, LC_{70}, LC_{90}, LC_{99} nano titanium dioxide was calculated during times of 24, 48, 72 and 96 hours. The results of this study show $LC50_{96h}$ nano titanium dioxide for goldfish is 19985.34±2.27 ppm and the maximum allowable concentration (MAC Value) calculated for this toxin is 1998.534 mg/L.

The blood factors in 50% LC_{50} concentrations of nano-titanium dioxide are presented in Table 2. No mortality observed in the control group. Fish exposed to nano-titanium dioxide for seven days, a significant change in hematological (Hb, MCH, MCHC, MCV, RBC), and immunological (WBC) indices concentrations occurred compare to the control groups ($p<0/05$). In 50% LC_{50} concentrations of nano-titanium dioxide, WBC parameter showed significant reduction toward control group ($P<0.05$). On the other hand, RBC factor significantly increased toward control ($P<0.05$). MCV, Hb and MCHC did not show significant changes toward control group ($P>0.05$). HCT blood factor showed significant increase toward control ($P<0.05$).

Also, Horsefall and Spiff (1998) showed that the extent of evacuation of oxygen in the water is often an action of the concentration of the organic pollutant in it. Fayeofori et al. (2012) showed that from the international assortment of toxicity of material based on their mean mortal dose the water dissolved deduction of diesel fuel is slightly toxic to *P. koelreuteria*. The exposure of *P. koelreuteria* to water-soluble fraction of diesel fuel showed mortality even at low concentrations. This agrees with earlier reports on the effect of water-soluble components of hydrocarbon on aquatic life (Oladimeji et al. 1988; Dede et al. 2001; Fafioye et al. 2006).

Table 1. Lethal Concentrations (LC_{1-99}) of nano titanium dioxide (mean ± Standard Error) depending on time (24-96h) in goldfish

Amount	24 hours	48 hours	72 hours	96 hours
LC_1	17649.49±5.77	16168.40±3.84	14603.12±2.52	**14097.20±2.27**
LC_{10}	21434.13±5.77	19327.38±3.84	17342.47±2.52	**16741.65±2.27**
LC_{30}	24176.82±5.77	21399.34±3.84	19327.64±2.52	**18658.05±2.27**
LC_{50}	**26076.39±5.77**	**22921.64±3.84**	**22077.56±2.52**	**19985.34±2.27**
LC_{70}	27975.97±5.77	24443.94±3.84	22077.49±2.52	**21312.63±2.27**
LC_{90}	30718.65±5.77	26641.90±3.84	24062.66±2.52	**23229.03±2.27**
LC_{99}	34503.30±5.77	29674.88±3.84	26802.01±2.52	**25873.48±2.27**

Table 2. Hematological indices of goldfish exposed to nano titanium dioxide at 50%LC50 dose

	RBC	Hb	HCT	WBC	MCH	MCHC	MCV
Control	0.58±0.00	5.73±0.08	15.73±0.06	15033.00±88.19	97.59±0.27	33.18±0.01	263±1.45
50%LC_{50}	0.61±0.00	5.76±0.08	18.33±0.08	14500.00±57.73	97.53±0.76	33.15±0.45	297±0.00

Khabbazi and et al showed, No mortality was observed after (0 - 96 h) of exposure. The analysis of hematological factors showed that Copper oxide nanoparticles affected the counts of white blood cells, eosinophils, lymphocytes, hematocrits, neutrophils, MCHC, MCV and MCH and did not have any effects on hemoglobins and monocytes. The information showed that the throughout hardness 270 ppm thwarted the lethal effect of copper on rainbow trout and no mortality was recorded (Khabbazi et al. 2015). The $LC50_{96h}$ values of Copper oxide nanoparticles was 2.19±0.003 mg/L. *Rutilus rutilus* exhibited significantly lower Hb, Hct and RBC values and a significant increase in the MCH numbers, WBC, MCV and MCHC indicator ($p<0.05$). Reduce glucose and increase cortisol content in blood plasma were observed in the fish exposed to Copper oxide nanoparticles than those in control group ($p<0.05$). These alterations indicate *Rutilus rutilus* sensitivity to CuO nanoparticles and changes in blood factors would be a useful tool for measurement early exposure to CuO NPs (Jahanbakhshi et al. 2015).

Singh and Srivastava (2010) showed the oxygen transport and carbon dioxide in the blood are delicate and complex. The status acid-base and electrolyte are dependent on red blood cells, as a result. The RBC reduction in blood cause respiratory limitations. In fact, one of the reasons for the change in the number of blood RBC, the accumulation of red blood cells in the gills of the fish is under stress pollution and reducing theirs in the blood (Narain and Srivastava 1989). In this study results showed $LC50_{96h}$ nano titanium dioxide for goldfish is 19985.34±2.27 ppm and the maximum allowable concentration (MAC Value) was calculated for this toxin 1998/534 mg/L.

Singh and Sirvastava (1989) showed the change in hemoglobin could be due to changes in the number of red blood cells. Since hemoglobin in red blood cells can be affected by RBC disorders. For example, hemoglobin reduction can be the result of the rupture of red blood cells. In 50% LC_{50} concentrations nano-titanium dioxide, WBC parameter showed significant reduction toward control group. But RBC factor significant increase toward control. MCV, Hb and MCHC are not showed significant changes toward control group. HCT blood factor showed significant increase toward control. Mean bulk of red blood cells by apportioning the Hb is expressed as MCV. Since The MCH, MCV, MCHC quantities are calculated based on Hb and HCT content and RBC number, changes in these factors will lead to changes in MCV, MCH and MCHC values (Desai et al. 2012).

Who stated increase in WBC numbers may be happening to dominate stressful condition. Significant reduction in plasma glucose levels after exposure to stressors has reported by few authors. A considerable decrease in glucose content of pesticide-treated *Channa punctatus* (Agrahari et al. 2007). Decreased hemoglobin and HCT content may be ascribed to the stress induced by feeding during the test, collapse of erythrocytes as a result of poison stress. There was significant change in hematological (Hb, MCH, MCHC, MCV, RBC), and immunological (WBC) indices concentrations toward the control groups. The results of this study showed nano titanium dioxide can have a negative effect on blood indices in goldfish, and reduces red blood cells and hemoglobin in fish, eventually caused the death of fish. Physiological response to nanoparticles is different in fish species. In toxicity, nonmaterial's should consider the possibility adverse effects of chronic toxicity. According to this study, development of nanotechnology and use of products of this technology in various industries can damage marine ecosystems. The results of this study showed, however, LC_{50} of nano titanium dioxide was very high that means it doesn't have much mortality effect, but it has a negative effect on blood indices in goldfish and reduces red blood cells and hemoglobin in fish, eventually caused the death of fish.

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