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Blood biochemical changes in common carp (*Cyprinus carpio*) upon co-exposure to titanium dioxide nanoparticles and paraquat

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Abstract

Research on eliminating organic pollutants in water by using heterogeneous photocatalysts such as nano-TiO_2 abound. However, the question is whether metabolites, resulting from optical dispersion of environmental pollutants, are still toxic to aquatic organisms. Therefore, this study was designed to evaluate the effect of a co-exposure to paraquat and TiO_2-NPs on blood biochemical indices of common carp. Fish were exposed to 0.2 and 0.4 mg L^{-1} paraquat with 0.125 mg L^{-1} TiO_2-NPs for 21 days under different light conditions, including natural photoperiod (16 L $8h^{-1}$ D) and complete darkness. No significant alterations in the Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Lactate dehydrogenase (LDH), Alkaline phosphatase (ALP) and Creatine phosphokinase (CPK), activities and total protein, albumin, globulin, glucose, cholesterol and triglyceride levels were observed in fish exposed to TiO_2-NPs and 0.2 mg L^{-1} paraquat under normal lighting conditions. However, a significant change in blood biochemical indices in fish exposed to TiO_2-NPs with 0.4 mg L^{-1} paraquat (16 L $8h^{-1}$ D) and in fish exposed to TiO_2-NPs and paraquat (under darkness). A significant decrease in the activity of Gamma-glutamyltransferase (GGT) and a significant increase in creatinine level were observed in all groups which were exposed to TiO_2-NPs and paraquat. The results of this study indicate that using 0.125 mg L^{-1} nano-TiO2 in order to remove paraquat (0.2 mg L^{-1}) under lighting conditions can minimize the adverse effects of paraquat and its metabolites on blood biochemical indices of fish. So, using nano-TiO2 (0.125 mg L^{-1}) to remove paraquat under lighting conditions can significantly reduce its toxic effects.

Keywords: Photocatalyst, TiO_2, Paraquat, Biochemical parameters, Common carp.

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Introduction

Paraquat is one of the most widely used herbicides in the world. By disturbing the photosynthesis process, this herbicide affects all exposed green parts of the plants. Paraquat interacts with electrons produced in chloroplast and produces free radicals, leading to damaged cell membranes and damaged plant tissues (Vidović *et al*., 2016). It is also highly toxic to animals and human beings and acts through lipid peroxidation by reducing free radicals such as superoxide ($-O_2$ •), hydroxyl (-OH •) and hydrogen peroxide (H_2O_2) which have a significant role of damaging cell membranes (Cantavenera *et al*., 2007; Banaee *et al*., 2013; Jebali *et al*., 2013; Khalighi *et al*., 2016; Vidović *et al*., 2016).

Paraquat has been applied in farms to control weeds for over 40 years. It is found in soil and surface waters as an environmental pollutant due to its widespread usage (Vidović *et al*., 2016). The results of our previous study indicated paraquat's potential to jeopardize the health of common carp by making alterations in their blood biochemical parameters (Banaee *et al*., 2016a).

Therefore, in order to prevent environmental pollution, we must look for ways to remove these compounds from the environment. Among different alternatives, developing technologies which convert high amounts of toxic compounds to non-toxic ones has attracted researchers' attention (Zahedi *et al*., 2015; de Figueiredo-Filho *et al*., 2017).

Using heterogeneous photocatalysts can be effective in oxidation and decomposition of many organic materials in surface waters. These compounds oxidize organic materials by producing (OH •) radicals in several phases (Lachheb *et al*., 2011). Using the semiconductor TiO2 as a catalyst for the photo-oxidation of organic materials has been of interest in recent years (Fenoglio *et al*., 2009; Lachheb *et al*., 2011). TiO2 absorbs the sunlight and photocatalyzes the decomposition of organic and chemical compounds in water (Zahedi *et al*., 2015). TiO2 has also been used as a photocatalyst in wastewater treatment systems due to its high capability of destroying organic contaminants, low toxicity, insoluble property and high durability in water (Fenoglio *et al*., 2009; Lachheb *et al*., 2011; Arbab *et al*., 2012). Absorbing wavelengths lower than 380 nm by TiO2 resulted in exciting electrons in titanium atoms. The excited electrons leave the valence band and enter the conduction band, leading to the valence shell gaining electrons. The extra electron in the conduction shell interacts with organic materials in water and causes the production of free radicals. These free radicals are capable of oxidizing chemicals, redox reaction of metals and lipid peroxidation of membranes (Lachheb *et al*., 2011). Therefore, nano-TiO_2 is used in municipal and industrial wastewater treatments (Lachheb *et al*., 2011). However, TiO_2 can affect aquaculture. In our previous study, the sublethal toxicity of TiO_2 was investigated under different lighting conditions (Lee *et al*.,

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2012; Banaee *et al*., 2016b). The question is whether application of nana TiO2 to remove environmental pollutants reduces their toxic effects or raises the toxicity of other pollutants. Thus, this study makes a biological evaluation of toxic effects of intermediate compounds formed during or after paraquat and nano-TiO_2 treatments. Conventionally, fish are commonly used in assessing the impact of environmental pollutants in aquatic ecosystems. Therefore, this study also uses common carp as a bio-indicator. Based on this hypothesis, changes in blood biochemical parameters of fish which were exposed to paraquat, and probable optical dispersion of paraquat and TiO_2 nanoparticles were studied.

Materials and methods

Chemical materials

Paraquat (Gramoxone), 20%, was obtained as a commercial preparation (Jiangsu Hai Bang Pharmaceutical Co., China, imported by Iran). All biochemical kits were purchased from Pars Azmun Co, Iran. Commercial TiO_2 nanoparticles, with an average primary particle size of 20 nm in the form of a powder (Table 1), were purchased from Iranian Nano-materials Pioneers Company, Iran (Figs. 1,2,3).

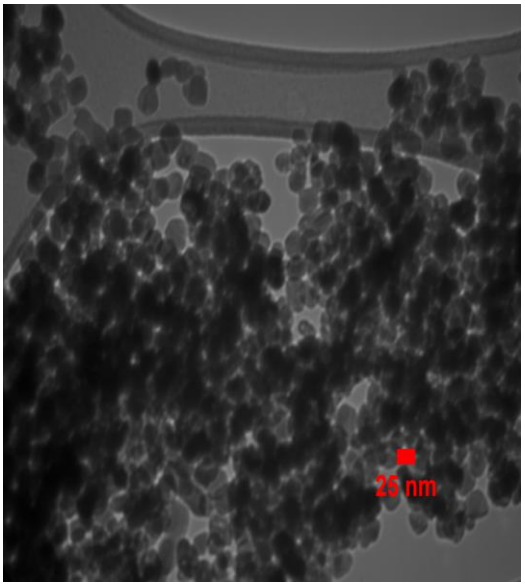


Figure 1: TEM micrographs of the Nano-TiO_2 powders (Adapted from the catalog of Iranian Nano-materials Pioneers Company).

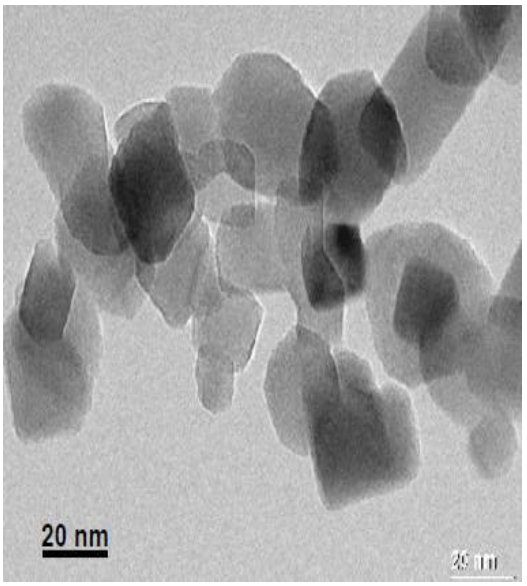


Figure 2: SEM micrographs of the Nano-TiO_2 powders (Adapted from the catalog of Iranian Nano-materials Pioneers Company).

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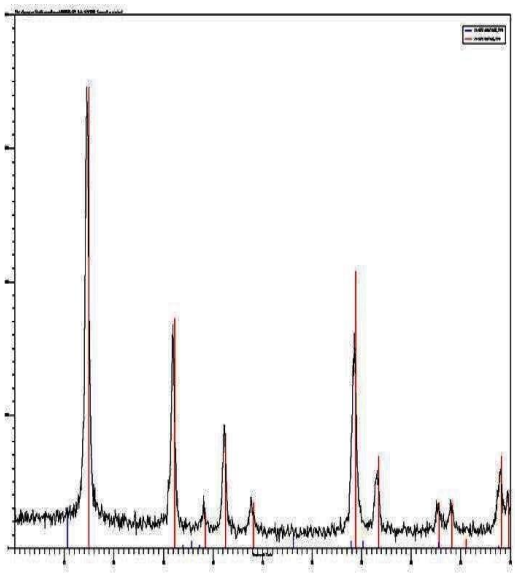


Figure 3: The X-ray powder diffraction (XRD) curves of Nano-crystalline TiO_2 (Adapted from the catalog of Iranian Nano-materials Pioneers Company).

Fish

Juvenile common carp, *Cyprinus carpio*, with a body weight of 35±5 g were maintained in the Department of Aquaculture, Khatam Al-anbia University of Technology, Iran. The fish were maintained in 80 L tanks in de-chlorinated tap water under controlled environmental conditions at 24±2 °C on a 16:8 h (light: dark) photoperiod. Fish were fed with commercial carp pellets (Beyza Fedd Mill Co. Iran) at the rate recommended by the manufacturer.

Preparing a stock solution of paraquat and TiO_2 nanoparticles

The Paraquat aqueous solution was prepared at concentrations of 400 and 800 mg L^{-1} and put into 1-liter Pyrex glass containers as photo-reactors. Before exposing the photo-reactors to light, nano-TiO2 solution (250 mg L^{-1}) was prepared and added to them after ultrasonication (10 min, 35 KHz, 100/400 W) using an ultrasonic bath (Elma, Germany), and was placed in a shaker for 24 hours in complete darkness (Li *et al*., 2002). Then, the 2 groups of photo-reactors were kept under normal lighting condition (16L/8D) and 2 other groups were kept in complete darkness. Before use, samples were again placed in a shaker for 30 minutes. Then, the solutions were added to dechlorinated tap water in exposure tanks, in order to obtain nominal concentrations of 0.125 mg L^{-1} TiO_2 and 0.2 mg L^{-1} or 0.4 mg L^{-1} paraquat.

Sub-lethal toxicity

One hundred and eighty common carp were randomly distributed in fifteen 80 L tanks (5 treatments with three replicates) and the experiment was run in the form of sub-lethal toxicity tests for 21 days. Fish maintained in tap water for 21 days were considered as the control group (group I). Specimens were exposed to a combined dose of 0.125 mg L^{-1} TiO_2-NP and 0.2 mg L^{-1} paraquat (group II) and/or 0.4 mg L^{-1} paraquat (group III) under normal photoperiod conditions (16 L/8 D). Group IV and V were exposed to a combined dose of 0.125 mg L^{-1} TiO2-NP and 0.2 mg L^{-1} paraquat and/or 0.4 mg L^{-1} paraquat respectively, under continuous dark conditions (0 L/24 D). Sub-lethal concentrations were selected according to previous studies re done by Banaee *et al.* (2016a) and Banaee *et al.* (2016b);.

Hence, to evaluate the toxicity of TiO_2-NPs and paraquat, the current study was carried out under normal

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photoperiod (16 L/8 D) conditions and absolute darkness conditions. Visible light irradiation was provided by four white fluorescent lamps (600 lux, 40 W, Pars-Shahab, Iran). In the darkness experiments, tanks were wrapped with opaque sheets and were kept in the dark without the intervention of any visible light. Therefore, the natural photoperiod (16:8 h) condition was considered as photoperiod condition and darkness (no irradiation) was regarded as the dark condition throughout the experiment (Dalai *et al*., 2013).

Although continuous aeration of water may partly prevent deposition of nanoparticles on the bottom of tanks, nanoparticles tend to form agglomeration (Hao *et al*., 2009). The actual amount of TiO_2 NPs may decrease up to 50% after 3 days (Hao *et al*., 2009). Therefore, 50% of the water must be exchanged every 12 hours and nanoparticles solution and the herbicide should be added again to the tanks to maintain TiO_2-NPs and paraquat concentrations constant (equivalent to 0.125 mg L^{-1} TiO_2-NP, 0.2 and 0.4 mg L^{-1} paraquat, respectively). This is necessary to inhibit the agglomeration of NPs and their absorption by fish feces and uneaten food.

Table 1: Physicochemical proprieties of TiO_2 nanoparticles according to the Iranian Nano-materials Pioneer.

Titanium Oxide	**TiO_2, 80 vol% anatase+ 20 vol% rutile**
Purity	+99%
Average primary particle size (D50)	20 nm
Specific surface area (SSA)	10-45 m^2 g^{-1}
Color	White
Bulk density	0.46 g ml^{-1}
pH	5.5-6.0
TiO_2	≥99%
Al	≤17 ppm
Mg	≤65 ppm
Si	≤120 ppm
Ca	≤75 ppm
S	≤130 ppm
Nb	≤80 ppm
Loss of weight in drying	0.48%
Loss of weight on ignition	0.99%

Sampling and analysis of blood biochemical parameters

Fish were starved for 24 h before sampling. After the 21-day exposure period, 18 fish from each treatment (6 fish from each tank) were taken out for sub-lethal toxicity studies. Fish were quickly netted from tanks and placed in 4 liter buckets filled half with 200 mg L^{-1} of clove powder solution. Blood samples were collected from the caudal vein using heparinized syringes, centrifuged at 6000 × g for 10 min and stored at -25 °C.

Total plasma protein and albumin, (Johnson *et al*., 1999), glucose (Sacks, 1999), cholesterol and triglyceride (Rifai *et al*., 1999), and creatinine

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levels (Foster-Swanson *et al*., 1994) were measured using commercially available kits from Pars Azmun Company, Tehran, Iran. The plasma globulin was estimated based on the ratio of albumin versus total protein.

The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and creatinine kinase (CK) (Moss and Henderson, 1999) were measured in plasma using commercially available kits from Pars Azmun Company, Tehran, Iran. All biochemical parameters were measured using a UV/VIS spectrophotometer (model Biochrom Libra S22).

Statistical analysis

All data were examined for normality (Kolmogorov-Smirnov test) and then analyzed for significance using one-way analysis of variance (ANOVA). The significant means were compared by Duncan's test and a $p<0.05$ was considered statistically significant. Statistical analyses were performed using SPSS 19 (IBM Corp.). Data are presented as mean±SD.

Results

Although no significant changes were observed in AST activity between fish exposed to nano-TiO2 with 0.2 mg L^{-1} paraquat (under normal lighting) and the control group, a significant decrease was observed in AST activity in plasma of fish, simultaneously exposed to nano-TiO_2 and 0.4 mg L^{-1} paraquat (under normal lighting conditions) and of fish exposed to nano-TiO_2 and 0.2 mg L^{-1} paraquat (under darkness). However, AST activity in fish exposed to nano-TiO_2 and 0.4 mg L^{-1} paraquat (under darkness) was significantly higher than that of the control. The results indicated a significant increase in ALT, LDH, ALP and CPK in fish exposed to nano-TiO_2 and 0.4 mg L^{-1} paraquat (under lighting conditions) compared to the control. No significant differences were found in ALT, LDH, ALP, and CPK activity in fish exposed to nano-TiO_2 and 0.2 mg L^{-1} paraquat (under lighting conditions) and the control group. The activity of ALT, LDH, ALP, and CPK in fish exposed to nano-TiO_2 and paraquat (under darkness) showed a significant increase compared to the control (Table 2).

GGT activity in fish exposed to nano-TiO_2 and paraquat indicated a significant decrease compared to the control group (Table 2).

A significant increase was found in plasma glucose levels in fish treated with nano-TiO_2 and paraquat (under darkness) and in fish exposed to nano-TiO_2 and 0.4 mg L^{-1} paraquat (under lighting conditions) compared to the control group (Table 2).

A significant increase was found in plasma creatinine levels in fish exposed to nano-TiO2 and paraquat compared to the control (Table 2).

The results indicated a significant decrease in levels of total protein, globulin and plasma cholesterol of fish exposed to nano-TiO_2 and paraquat (under darkness) as compared to the control (Table 2).

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Compared to the control group, a significant reduction in albumin and triglyceride levels was observed in plasma of fish which were exposed to nano-TiO_2 and paraquat (under darkness) and fish exposed to nano-TiO2 and 0.4 mg paraquat (under lighting conditions), (Table 2).

Table 2: Alterations in the blood biochemical parameters of common carp, *Cprinus carpio* exposed to sub-lethal concentrations of paraquat (0.2 and 0.4 mg L^{-1}) and TiO_2 nanoparticles (0.125 mg L^{-1}) under photoperiods (light: dark: 16: 8h) and dark (no irradiation) conditions.

Biochemical parameters	Control	TiO_2+0.2 mg Par (light)	TiO_2+0.4 mg Par (light)	TiO_2+0.2 mg Par (Dark)	TiO_2+0.4 mg Par (Dark)
AST (U L^{-1})	46.43±3.96^{b}	47.05±3.11^{b}	28.97±8.05^{a}	30.70±7.56^{a}	79.40±5.71^{c}
ALT (U L^{-1})	14.81±1.93^{a}	16.16±2.62^{a}	25.00±2.49^{b}	22.28±4.26^{b}	22.09±5.01^{b}
LDH (U L^{-1})	154.29±11.91^{a}	185.74±16.40^{a}	257.67±47.78^{b}	243.79±42.16^{b}	357.94±75.67^{c}
ALP (U L^{-1})	60.51±5.91^{a}	61.00±4.44^{a}	77.40±9.94^{b}	122.00±17.47^{c}	148.33±25.20^{d}
CPK (U L^{-1})	328.47±78.59^{a}	367.31±58.33^{a}	735.52±127.02^{b}	1071.78±113.40^{c}	1189.19±92.19^{c}
GGT (U L^{-1})	14.29±1.07^{c}	10.04±0.93^{b}	6.78±1.17^{a}	5.94±0.84^{a}	5.82±1.05^{a}
Glucose (mg L^{-1})	61.82±10.35^{a}	58.23±3.18^{a}	76.47±9.91^{b}	78.31±6.08^{b}	84.13±14.94^{b}
Creatinine (mg L^{-1})	0.15±0.02^{a}	0.80±0.13^{b}	1.25±0.54^{c}	1.36±0.39^{c}	1.11±0.22^{bc}
Total protein (g dL^{-1})	4.10±0.35^{b}	3.75±0.51^{b}	3.86±0.61^{b}	2.54±0.22^{a}	2.89±0.52^{a}
Albumin (g dL^{-1})	2.05±0.28^{b}	1.86±0.30^{ab}	1.71±0.27^{a}	1.76±0.27^{a}	1.85±0.25^{a}
Globulins (g dL^{-1})	2.05±0.53^{b}	1.89±0.49^{b}	2.15±0.67^{b}	0.79±0.32^{a}	1.04±0.45^{a}
Cholesterol (mg L^{-1})	147.94±17.20^{b}	139.48±20.79^{b}	133.64±13.86^{b}	96.69±17.26^{a}	90.63±24.98^{a}
Triglycerides (mg L^{-1})	225.08±16.78^{c}	229.13±24.06^{c}	137.09±9.71^{b}	140.85±12.32^{b}	79.97±9.75^{a}

Significant differences between values when compared with control groups were characterized by alphabet symbol (p<0.05). Values represent mean±S.D.

Discussion

Paraquat solution has a dark green color. Optical dispersion of this solution in the presence of nano-TiO_2 was visible based on the change of color during 21 days of the experiment. However, in spite of TiO_2 nanoparticles, there was a minor change of paraquat color in the photoreactor which was kept in complete darkness. Zahedi *et al*. (2015) approved optical dispersion of paraquat in the presence of TiO2 nanoparticles under different lighting conditions.

A significant decrease in AST activity can be due to an increased rate of nicotinamide adenine dinucleotide phosphate (NADPH) consumption during fish exposure to 0.4 mg L^{-1} paraquat and nano-TiO_2 (under normal lighting conditions). Moreover, an increase in levels of final metabolites such as creatinine, urea, and ammonia, as a result of protein breakdown inhibits the activity of this enzyme (Sette and de Almeida Lopes, 2014). Finally, lack of pyridoxine, as an essential cofactor in AST synthesis is another reason for reduced AST activity in blood of fish. On the other hand, a significant increase in AST activity in fish exposed to nano-TiO_2 and 0.4 mg L^{-1} paraquat (under darkness) may suggest severe damage to the liver. Increased AST

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activity was found in fish exposed to 0.4 mg L^{-1} paraquat (Banaee *et al*., 2016a).

A significant increase in ALT, LDH, ALP and CPK activity in fish exposed to TiO2 nanoparticles and 0.4 mg L^{-1} paraquat (under lighting conditions) can be the result of damage to cells. Therefore, alterations in the activity of these enzymes are a sensitive biomarker for damage to cell membranes (Gholizadeh Zare Tavana *et al*., 2018). Increased activity of ALT, LDH, ALP and CPK is reported in fish exposed to sublethal concentrations of paraquat (Nwani *et al*., 2015; Banaee *et al*., 2016a) and TiO_2 nanoparticles under different lighting conditions (Banaee *et al*., 2016b). LDH is a cytoplasmic enzyme which is found in all cells. It has an important role in cellular metabolism and converting pyruvate to lactate. In hypoxia, LDH catalyzes glycolysis in the absence of oxygen through re-oxidation of NADH by lactate (Murray *et al*., 2003). The release of lactate from red blood cells, skeletal muscle cells and liver cells to the blood after fish exposure to environmental pollutants can increase LDH activity in plasma. A significant increase in ALP activity in fish exposed to nano-TiO_2 and paraquat indicates a disturbance in the activity of liver and gall bladder due to their role in paraquat detoxification and excreting its metabolites (Banaee *et al*., 2016a) or accumulation of titanium in liver (Shi *et al*., 2016). Similar results were reported in *C. carpio* exposed to dimethoate (Rezaei Shadegan and Banaee, 2018). Creatine phosphokinase is an intracellular enzyme which is found in striated and smooth muscles and the brain. This enzyme has a key role in regulating the high energy needed in the production and consumption of phosphate in tissue contraction (Soleimany *et al*., 2016). Physiologically, CPK exists in plasma which indicates constant release of this enzyme from muscle cells (Goicoechea *et al*., 2008). The increased activity of CPK in plasma of fish exposed to nano-TiO_2 and paraquat can be attributed to the destruction of muscle fibers, toxic damage or changes in the enzyme activity or structural proteins. Lack of a significant difference in ALT, LDH, ALP and CPK activity in fish exposed to nano-TiO_2 and 0.2 mg L^{-1} paraquat (under lighting conditions) and the control group indicates optical dispersion of paraquat and removal of its metabolites.

Gamma-glutamyl transferase (GGT) is attached to the cell membrane and has a role in the metabolism of glutathione (GSH). Therefore, GGT has an important role in maintaining cellular antioxidants and making a balance in proliferation processes or programmed cell death (Wen *et al*., 2017). Thus, a significant decrease in GGT activity in fish treated with nano-TiO2 and paraquat may suggest the occurrence of oxidative stress. Decreased activity of GGT is reported in fish exposed to sub-lethal concentrations of paraquat (Banaee *et al*., 2016a) and nano-TiO_2 under different lighting conditions (Banaee *et al*., 2016b). Similar results were observed in *C. carpio* exposed to

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chlorpyrifos and polyethylene glycol (Hatami *et al*., 2019).

Jin-hui, (2012) indicated that TiO_2 could interact with water molecules after absorbing light leading to peroxidation of organic material by producing ROS. Under lighting conditions we expect TiO_2 to increase the rate of optical dispersion of paraquat, compared to the darkness condition. However, an increase in paraquat concentration (0.4 mg L^{-1}), metabolites of paraquat and TiO_2 can have adverse effects on the activity of AST, ALT, LDH, GGT and CPK. Under darkness, TiO_2 can also interact with organic molecules by producing ROS (Fenoglio *et al*., 2009). Since paraquat breakdown is highly dependent on the lighting condition, despite TiO_2 presence and ROS production, the rate of optical dispersion of paraquat is very low in complete darkness. Therefore, the effects of paraquat toxicity and TiO_2 nanoparticles in dark conditions have increased damage to cell membranes. Similar results were observed in adult Wistar rats after exposure to TiO2 nanoparticles (Amara *et al*., 2013).

Fish exposure to nano-TiO_2 and paraquat (under darkness) can increase catecholamine and break down glycogen storage in liver and muscles (Narra *et al*., 2015), leading to a significant increase in plasma glucose level in fish. A significant increase in glucose was also found in sub-lethal concentrations of paraquat (Nwani *et al*., 2015; Banaee *et al*., 2016a). Blood glucose in fish exposed to nano-TiO_2 increased by the reduction of liver glycogen storage and the increase of glucose release to the blood (Liu *et al*., 2009; Lourenço, 2012; Banaee *et al*., 2016b). These results agree with a previous study carried out on mice exposed to TiO_2 nanoparticles (Duan *et al*., 2010). However, no significant changes were found in glucose levels in fish treated with nano-TiO_2 and 0.2 mg L^{-1} paraquat, which can be attributed to paraquat optical dispersion during 21 days of the experiment. These findings are in accordance with Amara *et al*. (2013).

A significant increase in plasma creatinine in fish exposed to nano-TiO_2 and paraquat suggests renal damage. A significant increase in creatinine was also found in fish treated with sub-lethal concentrations of paraquat (Banaee *et al*., 2016a). Nephrotoxicity and increased creatinine level is reported in fish exposed to nano-TiO_2 (Banaee *et al*., 2016b; Wu *et al*., 2016). Similar results were reported in mice exposed to TiO2 nanoparticles (Duan *et al*., 2010).

Protein is the most important organic material needed to construct and repair tissues and has an important role in providing energy for fish (Binukumari *et al*., 2017). In fish, protein breakdown in order to provide energy and to deal with toxic effects of paraquat, intermediate metabolites, and a combination of TiO_2 nanoparticles and paraquat (under darkness) may explain the significant decrease in total protein of plasma. Reduction of total protein has a significant effect on albumin and globulin levels in plasma of fish exposed to nano-TiO_2 and paraquat. A

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significant decrease in total protein, albumin and globulin is also reported in fish exposed to sub-lethal concentrations of paraquat (Nwani *et al*., 2015; Banaee *et al*., 2016a) and nano-TiO_2 (Banaee *et al*., 2016b). A significant decrease in the total protein was also found in fish exposed to diazinon (Ahmadi *et al*., 2014) and paraquat (Nematdoost Haghi and Banaee, 2017).

A significant decrease in cholesterol and triglyceride levels in plasma of fish can be effective in yielding energy to cope with the toxicity of paraquat, intermediate metabolites, and a combination of nano-TiO_2 and paraquat. Paraquat and its metabolites or nano-TiO_2 may have significantly reduced cholesterol and triglyceride by disturbing the absorption of cholesterol and triglyceride in the intestine and disturbance in their biosynthesis due to hepatic damage. Similar results were found in fish exposed to different concentrations of paraquat (Banaee *et al*., 2016a). The results of our previous study indicated that exposure of fish to nano-TiO_2 under normal lighting conditions decreased cholesterol and triglyceride level. Nevertheless, these parameters were significantly increased in fish exposed to nano-TiO_2 under darkness conditions (Banaee *et al*., 2016b).

The results of investigating a combination of nano-TiO_2, paraquat, and its metabolites on biochemical parameters under different lighting conditions indicated that in spite of increased optical dispersion of paraquat in high concentration, its metabolites, primitive compounds, and TiO_2 can still be toxic to fish. In general, alterations in blood biochemical parameters of common carp, exposed to 0.125 mg L^{-1} nano-TiO_2 and 0.2 mg L^{-1} paraquat are similar to those of the control group. Therefore, using nano-TiO_2 (0.125 mg L^{-1}) to remove paraquat under lighting conditions can significantly reduce the toxic effects of paraquat and its metabolites. Nevertheless, alterations in biochemical parameters in plasma of fish exposed to nano-TiO_2 under darkness show the low rate of paraquat dispersion. Therefore, prior to discharging nano-TiO_2-treated wastewater to surface waters, we must make sure of the complete degradation of organic material to prevent the destructive effects of these compounds and their metabolites on fish health.

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