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ORIGINAL ARTICLE

Coexisting of titanium dioxide nanoparticles and diazinon on histopathology of common carp (*Cyprinus carpio*)

Ali Mansouri[1] · **Mokhtar Fathi**[2] · **Borhan Mansouri**[1,3] · **Nammam Ali Azadi**[4]

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Abstract The purpose of present study was to determine the sub-acute toxicity effects of diazinon on the gill and intestine tissues histopathology of common carp (*Cyprinus carpio*) in exposure to TiO_2 NPs. In this study, common carp (length: 21 ± 1.3 cm; weight: 11 ± 1.1 g) were assigned randomly to 12 experimental groups (15 each) and exposed to three non-lethal concentration of TiO_2 NPs, two non-lethal concentrations of diazinon, and six mixture concentrations of diazinon and TiO_2 NPs, as well a control group for a period of 14 days. For studies of classic histological, the samples of gill and intestine organs were prepared and stained by hematoxylin-eosin and, finally, was imagined by light microscopy. Compared to the control group, gill and intestine tissues of common carp in treatment groups, TiO_2, diazinon, and a mixture of TiO_2 and diazinon, showed higher histopathological alterations. The most commonly observed alterations in gill were hyperplasia, fusion of lamellae, aneurism, and necrosis. Common alterations in intestine were degeneration, increase in the number of goblet cells, increase in the number of lymphocyte, vacuolation, and necrosis and erosion. In conclusion, this research suggested that the coexistence toxicity of diazinon and TiO_2 NPs on the gill and intestine histopathology of common carp was higher than the diazinon and TiO_2 NPs alone.

Keywords Gill · Intestine · Histopathology · Co-exposure · Diazinon

Introduction

Organophosphorus pesticides are used in various sectors such as pest control in agriculture, forest, aquatic environments, and to protect human health and animals. Continuous and excessive use of pesticides threatens human health and other biological organisms, as well as pollution of water, soil, and air (Mirvaghefi et al. 2015). Hence, monitoring the natural environment for pesticides is most considered to determine their levels.

Diazinon is one of the organophosphate pesticides extensively used in pests of fruit trees, field and vegetable crops, lawns, and against soil insects (Banaee et al. 2011; Khoshbavar-Rostami et al. 2006). Reports published by the ministry of agriculture claim the annual consumption of diazinon in Iran to be 3775 tons (Bulletin of Agriculture Ministry of Iran 2008). Various studies have reported levels of diazinon and its derivates in rice paddy fields, sugar cane farms, and water resources of Iran, i.e., rivers located in north and south of Iran (Shayeghi et al. 2007; Arjmandi et al. 2010; Nasrabadi et al. 2011).

Diazinon can be absorbed by gills, gastrointestinal tract, and skin of organisms such as fish if it is present in the aquatic environment. Several studies have reported the toxic effects of short- and long-term exposure to sub-lethal diazinon on fish, including various biological and physiological aspects such as biochemical and hematological changes (Ghasemzadeh et al. 2015), oxidative stress (Mirvaghefi et al. 2015), growth performance (Khosravi-Katuli et al. 2014), immune response (Khoshbavar-Rostami et al. 2006), and histopathological alterations (Banaee et al. 2013).

✉ Borhan Mansouri
borhanmansouri@yahoo.com

1 Student Research Committee, Kurdistan University of Medical Sciences, Sanandaj, Iran

2 College of Agriculture, Payam Noor University, Tehran, Iran

3 Environmental Health Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran

4 Biostatistics Department, School of Public Health, Iran University of Medical Sciences, Tehran, Iran

Titanium dioxide nanoparticles (TiO_2 NPs) are used widely in cosmetic products, food additives, degrading organic contaminants, sunscreens, and personal care products among others. In the past decade, the use of TiO_2 NPs has grown rapidly. One consequence of this enormous use is the pollution of our aquatic ecosystems (Bourgeault et al. 2015). Although TiO_2 NPs have useful applications, but this nanoparticle has a toxic potential effect on living organisms (Vasantharaja et al. 2015). The toxicity mechanisms and behavior of nanoparticles in the presence of other environmental pollutants can alter the uptake of pollutants. Fang et al. (2015) reported an enhanced metabolism of pentachlorophenol in zebrafish larvae when TiO_2 NPs is present. They increased oxidative damages in early developing zebrafish larvae. Moreover, Zhu et al. (2011) illustrated that compared to tributyltin alone, the presence of 2 mg/L TiO_2 NPs increased the toxicity of Tributyltin up to 20-fold (*Haliotis diversicolor supertexta*) embryos. In another study, Wang et al. (2014) determined that TiO_2 NPs enhance BDE-209 (polybrominated diphenyl ether congener) bioavailability and metabolism, leading to thyroid endocrine disruption and developmental neurotoxicity in zebrafish larvae. To obtain comprehensive information in this area, more investigations are needed. Therefore, our study aimed to further investigate coexistence effects of diazinon and TiO_2 NPs on the gill and intestine histopathology of common carp (*Cyprinus carpio*) under controlled condition.

Materials and methods

TiO_2 NPs and characterizations

The TiO_2 NPs (anatase/rutile, 99 + , 20 nm) used in this study were purchased from Nanosany Co. (Mashhad, Iran). The purity, morphology, and the mean of unaggregated particle diameters of TiO_2 nanoparticles were determined by transmission electron microscopy (TEM) and scanning electron micrographs (SEM) (Fig. 1). Other characteristics of TiO_2 nanoparticles were 20 $m^2\ g^{-1}$ for SSA, 0.48 % for loss of weight in drying, 0.99 % loss of weight on ignition, 5.5–6.0 for pH, and 0.46 g cm^{-3} for bulk density. The hydrodynamic diameter and zeta potential of a 100 mg L^{-1} suspension of TiO_2 NPs in double distilled water were measured by dynamic light scattering (DLS) using a Zetasizer Nano (ZS) model ZEN3600 (Malvern Instruments Ltd., Worcestershire, UK). The hydrodynamic diameter and zeta potential of the TiO_2 NPs were 26.9 nm and −14.18 mV, respectively.

Test organism and experimental condition

Common carp (*Cyprinus carpio*) with mean length of 21 ± 1.3 cm and mean weight of 11 ± 1.1 g were obtained from a local aquaculture farm in northern Iran (Anzali city). Prior to the beginning of the experiment, they were acclimatized in 500-L tanks for 1 month supplied with continuously aerated tap water (21–28 °C) under 14 h daylight and 10 h darkness. Fish were fed up with commercially available fish food at a rate of 3 % body weight per day. Fish were deprived of food for 1 day prior to the experiment. The characteristics of the water used for the common carp exposures were pH 7.3 ± 0.54, conductivity 600 ± 10 μS/cm, hardness 5°dGH, temperature 24.0 ± 2 °C, and dissolved oxygen content (DO) 6.2 ± 0.4 mg/L.

For the future sub-acute experiments, non-lethal concentration of TiO_2 NPs and diazinon was chosen based on the published toxicity studies (Linhua et al. 2009; Lee et al. 2012; Ahmad 2011). Fish were assigned randomly to one control group and 12 experimental groups as follows: three non-lethal concentrations of TiO_2 NPs (5, 10, and 15 mg L^{-1}), two non-lethal concentrations of diazinon (0.1 and 1 mg L^{-1}), and six mixture concentrations of diazinon and TiO_2 NPs (0.1 plus 5, 10, and 15 mg L^{-1}; 1 plus 5, 10, and 15 mg L^{-1}). There were 15 fish at each group hence 180 common carp in total. For each group, exposure time was 4 days in an aquarium (60 L of water). Half of the water in the tank swapped with freshwater to keep concentration levels in daily basis. Moreover, during the exposure period, aeration was supplied to the tanks to prevent the propensity of aggregation.

Histological examinations

After the expiry of experimental period, of each respectively marked experimental group and the control group, four fish randomly selected and sacrificed. The gill and intestine organs of common carp were carefully removed and fixed in Boiun's fluid pending for further histology works. The fixed samples were transferred to formalin and kept for 24 h. The organs were dehydrated using a series of graded ethanol solutions, cleared in xylene, and embedded in paraffin wax. Slices of 5 μm were prepared from paraffin blocks by using a rotary microtome. These slices were then stained with hematoxylin-eosin and examined microscopically. The diameter and length of secondary gill lamellas as well as diameter of gill filaments were measured using the Axio Vision (Release 4.8.2). Samples used to assess three types of histopathological alterations after 96 h in the gill and intestine organs: none (−), mild (+), moderate (++), and severe (+++) effects. Moreover, the behavioral pattern was monitored regularly as suggested by Kumar et al. (2007).

Statistical analysis

The statistical package SPSS, version 16, (Chicago, IL, USA) was used for data analysis. For each gill parameter (diameters and lengths of secondary lamellae and diameter of gill

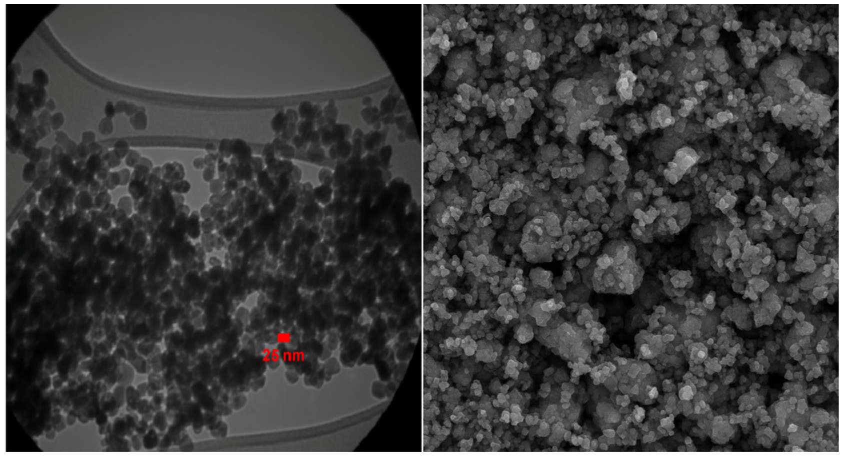


Fig. 1 TEM (left) and SEM (right) of tested TiO_2 nanoparticles

filaments), summary statistics were obtained by reporting mean ± SD. To compare the exposure effect of Diazinon, TiO_2 NPs, and their mixture on the gill parameters of common carp, one-way ANOVA was followed by the post hoc. To explore which treatment group differs significantly across each parameter, Tukey's HSD test was used. Data were inspected for normality assumption as well as the homogeneity of variance required by ANOVA. Ethical considerations and animal rights in this paper were considered, and the study was approved by Ethics Committee of the university (MUK.REC.1393.98).

Results

The alterations in behavior of common carp exposed to diazinon and TiO_2 NPs exhibited abnormal behavior such as hyperactivity, loss of balance, rapid swimming, increased surfacing activity, enhanced rate of opercular activity and convulsions with the changing groups of TiO_2 NPs and diazinon when compared to the control fish (Table 1**).** They become lethargic and mucous substance was secreted from the whole body. Moreover, the exposed fish swam to surface more often than the control fish; while neither mortality nor any visible changes in behavior was observed in the control group. In addition, abnormal behavior of common carp in the exposure to diazinon and TiO_2 NPs mixture was higher than the diazinon and TiO_2 NPs alone.

Table 1 Impact of TiO_2 NPs and diazinon on the behavior of common carp

Parameter	Control	TiO_2 NPs	Diazinon	TiO_2 NPs + Diazinon
Rate of opercular activity	−	+	+	++
Loss of balance	−	+	+	+++
Convulsions	−	+	++	+++
Rate of swimming	−	+	++	+++
Hyperactivity	−[a]	+	++	+++

[a] (−) none, (+) mild, (++) moderate, (+++) strong

Table 2 shows the mean and standard deviation of gill's parameters in each experimental group averaged over 10 samples. It appears that compare to the gill filaments of common carp in control group, the length of primary lamellae has been decreased in all treatment groups. The smallest mean length of gill filament, 17.44 μm, was observed in the group treated with mixture of TiO_2 NPs and diazinon. The 95 % confidence interval was (13.59, 21.28) which was significantly smaller than the mean length of primary lamellae in other groups (it has no overlap with other CIs). For the mixed group, primary and secondary diameter lamellae were also highest.

Figure 2 displays the trend of gill parameters among treated groups. The bars in the Fig. 1 represent 95 % confidence interval. There is no overlap between bars in the length and diameter of primary lamellae indicating significant differences in the gill characteristics due to exposure effect. In secondary diameter, the result is different somehow. The mixed group has inflated the secondary diameter of lamellae so that the mean diameter has increased significantly. Fish in mixed group experienced the most dramatic alterations in all gill parameters; they had shortest primary lamellae and thickest both primary and secondary lamellae diameters. The control group was characterized by longer gill filaments length and smaller lamellae diameters. The overall tendency was that gill filament length decreased with toxicity exposure, but primary and secondary diameter lamellae of common carp gills increased.

The one-way ANOVA of the length of gill filaments ($F = 51.11$, $p < 0.001$), primary diameter lamellae ($F = 53.11$, $p < 0.001$), and secondary diameter lamellae ($F = 56.97$, $p < 0.001$) were significant indicating meaningful differences between the mean of gill parameter among fish at groups under study. The 95 % confidence intervals (CIs) of this test are given by Fig. 3. Tukey HSD test adjusts for inflation of type I error as the number of two by two comparisons increase. This test

Table 2 Mean, standard deviation (SD), and 95 % confidence interval of gill's parameters for common carps at various experimental conditions

Gill parameter	Experimental group	Mean	SD	95 % confidence interval
Length of primary lamellae (L)	Control	49.55	3.88	(47.15, 51.95)
	Diazinon	26.09	4.98	(23.00, 29.17)
	TiO_2 NPs	34.07	8.22	(28.98, 39.17)
	Diazinon + TiO_2 NPs	17.44	6.20	(13.59, 21.28)
Diameter of primary lamellae (D1)	Control	13.78	2.13	(12.45, 15.10)
	Diazinon	36.25	7.50	(31.61, 40.90)
	TiO_2 NPs	19.87	3.81	(17.50, 22.23)
	TiO_2 NPs + Diazinon	48.54	10.60	(41.96, 55.11)
Diameter of secondary lamellae (D2)	Control	5.44	0.65	(5.03, 5.85)
	Diazinon	7.04	1.17	(6.31, 7.76)
	TiO_2 NPs	6.08	1.02	(5.44, 6.71)
	TiO_2 NPs + Diazinon	12.94	2.34	(11.49, 14.39)

marked all pair-wise comparisons of the mean length of gill filaments between treatments statistically significant at 5 % nominal level. Hence, treatment groups affect the length of primary lamellae in different manner. The largest difference in the length of primary lamellae was observed between mixed and control groups. For primary diameter, the only insignificant comparison observed between TiO_2 NPs and control treatments indicating no changes in primary diameter of gill when exposed to TiO_2 NPs. Exposure to other treatments altered the diameter of primary lamellae. As expected, for secondary diameter, the only treatment with significant effect was mixed group. All pair-wise comparisons between these treatments with others were highly significant whereas no statistically meaningful difference was observed between other treatments.

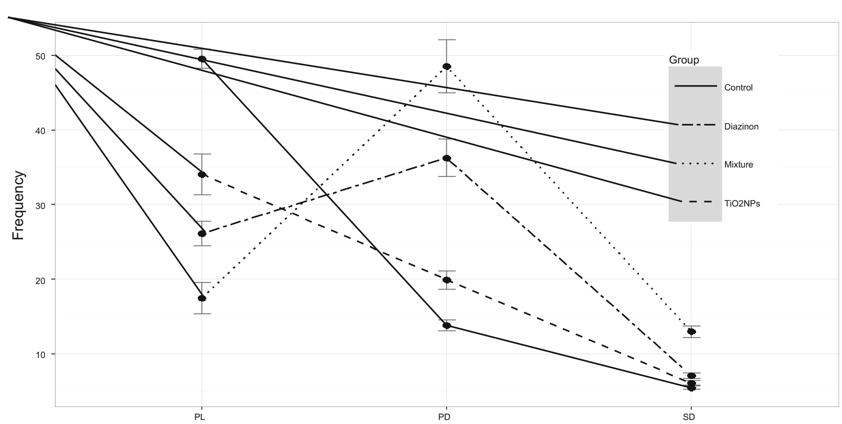
The results of histopathology alterations of gill and intestine organs of common carp at both control and experimental groups are displayed by Figs. 4 and 5, respectively. Compared to the control group, higher histopathological alterations were observed at treatment groups. The most common morphological changes found in the gill were dilated and clubbed tips (DCt), and hyperplasia (Hp) in groups treated with TiO_2 NPs; hyperplasia, fusion of lamellae (F), and lamellar synechiae (LS) in groups treated with diazinon; aneurism (An), hyperplasia, oedema (Oe), curvature (Cu), fusion of lamellae, epithelium shortening (ES), and necrosis (N) in groups treated with mixture of TiO_2 NPs and diazinon. The microscopical examination of the intestine in common carp revealed several histopathological changes such as degeneration (D), hemorrhage (Hm), increase in the number of goblet cells (INGC), expansion at villi structure (EVS), increase in the number of lymphocyte (INL), and necrosis and erosion (NE).

The results of this study indicated that the severity of gill and intestine alterations in the mixture of diazinon and TiO_2 NPs was more intense than those we observed at TiO_2 NPs and diazinon groups separately (Table 3). Gill tissue exposed to TiO_2 NPs exhibited initial injuries such as curvature, dilated, and clubbed tips before it extended to hyperplasia secondary lamella. In diazinon group, injuries were fusion of lamellae, hyperplasia, epithelium shortening, and lamellar synechiae. The severity of damages was deeper than injuries caused by TiO_2 NPs exposure. More excessive damages such as edema, aneurism, and necrosis were observed only in combination of diazinon and TiO_2 NPs. Moreover, results showed notable significant differences in gill parameters between different groups ($p < 0.05$; Table 4). It appears that the toxic effects of diazinon in gill tissues of common carp in the joint presence of TiO_2 NPs were synergistic.

Discussion

According to results, they become lethargic and mucous was secreted from the whole body of common carp and the exposed fish exhibited enhanced surfacing behavior. These observed behavioral alterations in NPs-treated fish are consistent

Fig. 2 Interaction plots with 95 % error bars for the length of primary lamellae (PL), primary diameter of gill lamellae (PD), and secondary diameter of gill lamellae (SD)



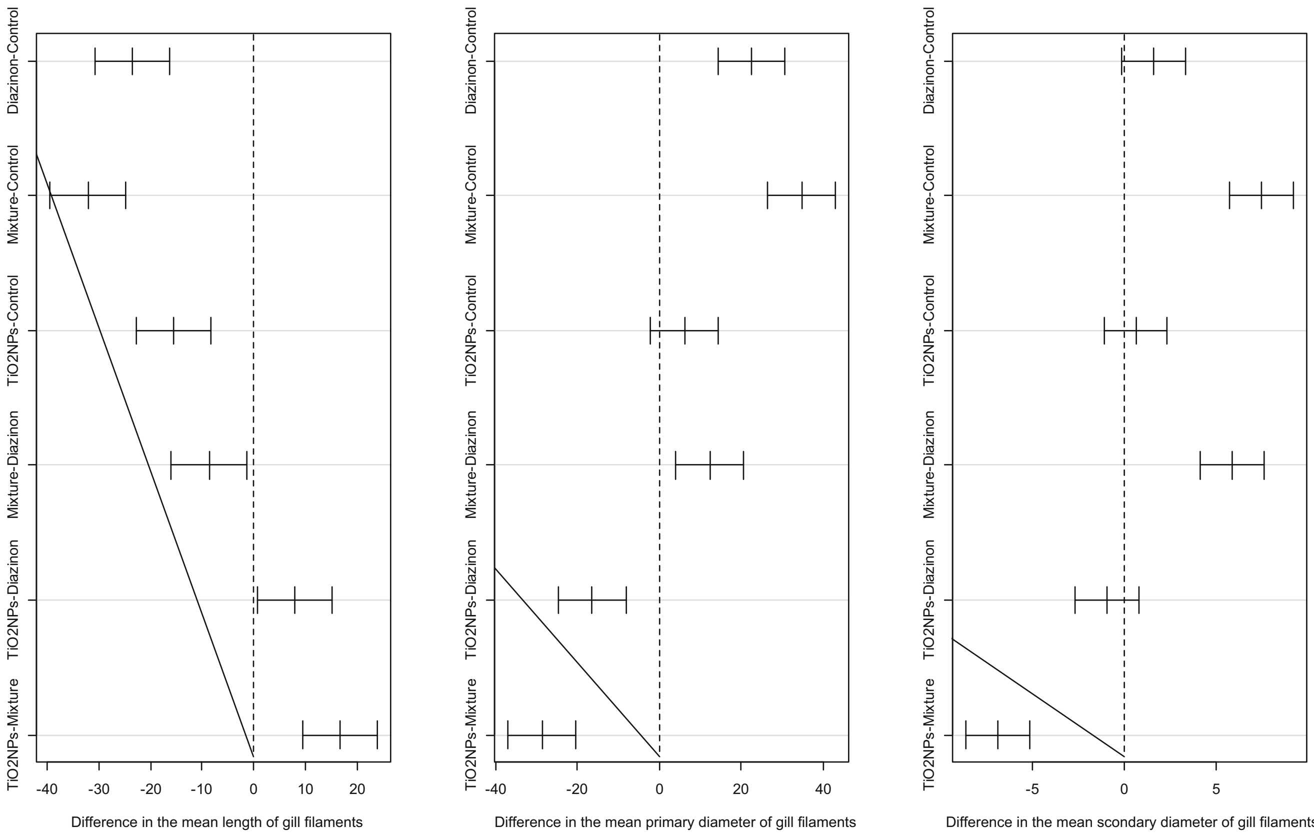


Fig. 3 95 % Tukey HSD pair-wise confidence intervals of differences in the mean levels of gill parameters at different treatment

with the previous reports on other nanoparticles (Begum et al. 2006) and other heavy metals (Olojo et al. 2005; Begum et al. 2006). For an aquatic organism such as fish, gills are vital organs for their multifunctional roles including respiratory organs and participate in many physiological activities, including respiratory, metabolites excretion, body fluid permeability balance, and acid–base regulation balance (Henry et al. 2012). Moreover, gills are directly in contact with exogenous toxicants in an aquatic environment (Nowrouzi et al. 2012; Baramaki et al. 2012; Majnoni et al. 2013). Previous studies suggest two types of gill damages: (i) type I; injuries such as hyperplasia and lamellae edema occurring due to defending mechanism of gill and (ii) type II injuries; direct injury such as necrosis and shedding of gill epithelium (Fanta et al. 2003; Cengiz and Unlu 2006). In the present study, we observed type I injuries in groups treated with either TiO_2 NPs or diazinon (i.e., hyperplasia, dilated and clubbed tips, curvature, epithelium shortening, and lamellar synechiae) and type II damages such as necrosis of gill epithelium observed in the mixed TiO_2 NPs and diazinon experimental groups indicating increased toxicity.

In this study, dilated and clubbed tips, curvature, epithelium shortening, and lamellar synechiae were observed in the gills after exposure to TiO_2 NPs, diazinon, and the mixture of TiO_2 NPs and diazinon. The similar histopathological anomalies that we observed have been also reported for other nanoparticles, i.e., Cu NPs on rainbow trout (Al-Bairuty et al. 2013), TiO_2 NPs on rainbow trout (Federici et al. 2007), cobalt (III) oxide (Co_2O_3) nanoparticle on zebrafish (Mansouri et al. 2015), and colloidal silver nanoparticle on zebrafish (Mansouri and Johari 2015). Dilated and clubbed tips as well as curvature in lamellar epithelium are one of the early histopathological anomalies reported as the effect of toxicant on aquatic species. In this case, the secondary lamella epithelium becomes inflammation by which the presences of nanoparticles reduce the useful surface gill and thus reduce their gill gas exchange.

One of the common changes in the gill of common carp exposed to different groups of TiO_2 NPs and diazinon were hyperplasia and fusion lamellae. In this condition, lamellae cell proliferation decreases the space between the lamellae leading to fusion of secondary lamellae (Banerjee 2007). Hyperplasia and fusion of lamellae observed more in the mixture group. This lesion was a defensive mechanism leading to a decrease in the respiratory surface and an increase in the toxicant-blood diffusion distance (Jaya and Shettu 2015). Lesions severity and extension of lamellar aneurysm and necrosis in gill tissue of common carp in mixed groups were higher than that we observed in either TiO_2 NPs or diazinon group. The formation of aneurysm is characterized by blood extravasations inside the lamellae and rupture of the pillar cell system, consequent dilatation of the blood vessels, or even the

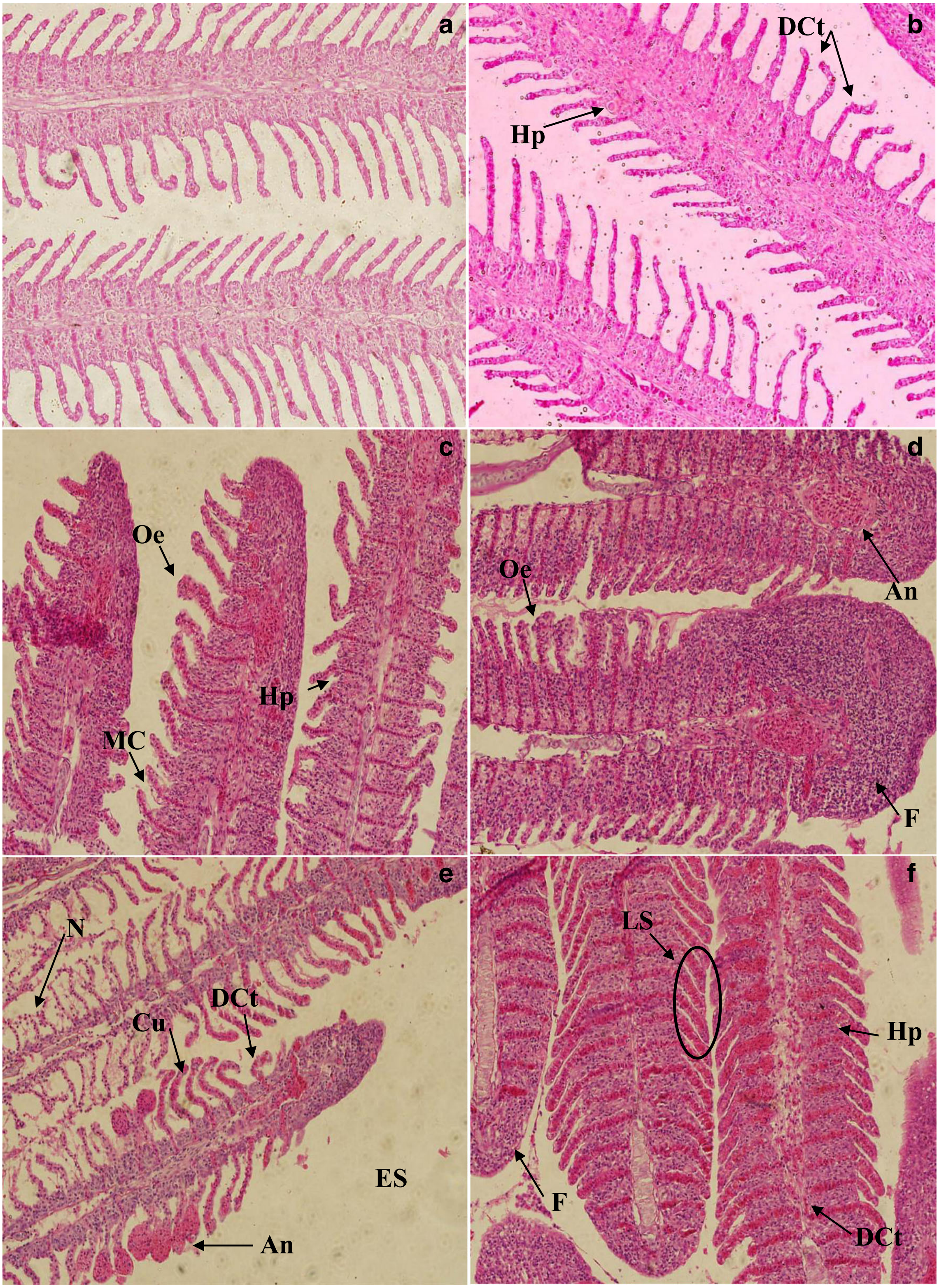


Fig. 4 Gill morphology in common carp in sub-acute period (14 days). The gills of control fish indicate only some small histopathological alterations (**a**), other treatment groups (**b** TiO_2 NPs; **c** diazinon; **d**, **e**, and **f** TiO_2 + diazinon) show injuries including aneurism (*An*), dilated and clubbed tips (*DCt*), hyperplasia (*Hp*), edema (*Oe*), curvature (*Cu*), fusion of lamellae (*F*), epithelium shortening (*ES*), dilated marginal channel (*MC*), lamellar synechiae (*LS*), and necrosis (*N*)

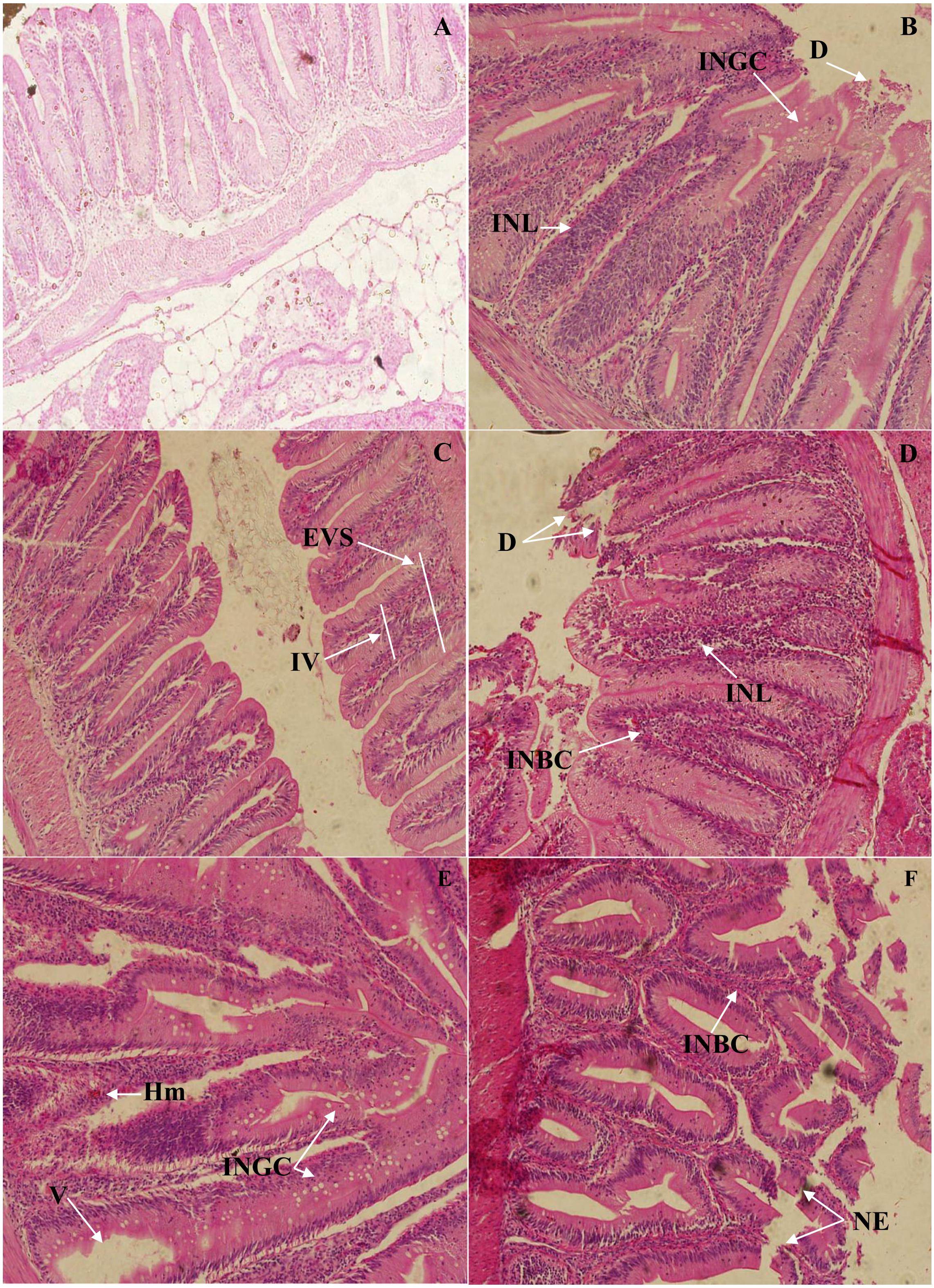


Fig. 5 Intestine morphology in common carp in sub-acute period (14 days). The intestine of control fish shows only some small histopathological alterations (**a**). Other treatment groups (**b** TiO_2 NPs; **c** diazinon; **d**, **e**, and **f** TiO_2 + diazinon) showed significant injuries including degeneration (*D*), integration of villi (*IV*), hemorrhage (*Hm*), increasing the number of goblet cells (*INGC*), expansion at villi structure (*EVS*), increasing the number of lymphocyte (*INL*), increasing the number of blood cells (*INBC*), vacuolation (*V*), and necrosis and erosion (*NE*)

Table 3 Summarized histopathological effects on the gill and intestine organs of common carp exposed to different groups of TiO_2 NPs, diazinon, and control fish

Organs	Damages								
Gill	An[a]	DCt	Hp	Oe	Cu	F	LS	ES	N
Control	−	−	−	−	+	−	−	−	−
TiO_2 NPs	−	+	+	+	+	+	−	−	−
Diazinon	+	+	++	+	−	+	+	−	−
TiO_2 NPs + Diazinon	++	+	+++	++	++	++	++	++	++
Intestine	D[b]	NE	INBC	IV	EVS	Hm	INGC	INL	V
Control	−	−	−	+	−	−	−	−	−
TiO_2 NPs	+	−	−	+	−	−	+	+	−
Diazinon	+	+	+	+	++	+	−	+	+
TiO_2 NPs + Diazinon	+++	++	++	+	++	++	++	+++	++

[a] *An* aneurism, *DCt* dilated and clubbed tips, *Hp* hyperplasia, *Oe* oedema, *Cu* curvature, *F* fusion of lamellae, *ES* epithelium shortening, *LS* lamellar synechiae, *N* necrosis

[b] *D* degeneration, *IV* integration of villi, *Hm* hemorrhage, *INGC* increase in the number of goblet cells, *EVS* expansion at villi structure, *INL* increase in the number of lymphocyte, *INBC* increase in the number of blood cells, *V* vacuolation, *NE* necrosis and erosion

direct effects of contaminants on these cells. In similar study, Mansouri et al. (2016) observed lesions of lamellar aneurysm and necrosis on the gill of zebrafish after acute exposure at combined of silver nanoparticles and mercury.

The intestine is the first organ that comes into contact with food-borne pollutants. The commonly observed alterations in intestine organs included an increase in the number of goblet cells, blood cells, lymphocyte, and vacuolation. The increase in number of goblet cells and increased mucus secretion are considered the first protective reaction to toxic agents and may temporarily reduce toxic impact (Handy and Maunder 2009). Similar alterations in the intestine organ of *Channa punctatus (Bloch)* exposed to Pesticide Endosulfan were reported by Haloi et al. (2013). Degeneration and necrosis and erosion in intestine organs were also observed in the present study and were reported by Cengiz et al. (2001) and Smith et al. (2007) for mosquitofish and rainbow trout exposed to single walled carbon nanotubes and endosulfan, respectively. Pereira et al. (2013) showed correlations between some histopathological responses and the ecological status reflecting the existence of a pollution gradient. According to our results, the severity of injuries in intestine organs was more intense in common carp treated with the mixture of diazinon and TiO_2 NPs.

Table 4 The secondary lamellae length, diameter, and gill filament diameters (μm) reported as mean (±SD) for different studied groups. One way ANOVA was used to compare the mean level of each parameter across groups

Groups	Gill		
	Diameter of gill filaments	Diameter of secondary lamellae	Length of secondary lamellae
Control	13.7 ± 2.1[a]*	5.4 ± 0.6[a]*	49.5 ± 3.8[a]*
TiO_2 NP_s	19.8 ± 3.8[a]	6.1 ± 1.1[a]	34.1 ± 8.2[b]
Diazinon	36.2 ± 7.4[b]	7.1 ± 1.1[b]	26.1 ± 4.9[c]
TiO_2 NPs + Diazinon	48.5 ± 10.6[c]	12.9 ± 2.3[c]	17.4 ± 6.2[d]
*p value***	<0.01	<0.01	<0.01

In each column, the numbers with different superscript letters differ significantly ($p < 0.05$)

*In each column, the values are statistically significant at $p < 0.05$

**One-way ANOVA: significant at $p < 0.05$

Diazinon was extensively used as an effective organophosphorus pesticide in agricultural activities and had been proven to have harmful effects in the organs of human and other organisms. So, the degradation of diazinon had attracted great attention, and recently, TiO_2 NPs have been tested to degrade the organic or inorganic compounds. Several studies have been reported in interaction between effects of TiO_2 NPs with heavy metals such as lead acetate (PbAC) in mice (Zhang et al. 2010), arsenic (As) in *Ceriodaphnia dubia* (Wang et al. 2011), cadmium chloride ($CdCl_2$) in human embryo kidney (HEK293T) cells (Xia et al. 2011), humic acid (HA) and Cd in zebrafish (Hu et al. 2011), and copper (Cu) on *Daphnia magna* (Fan et al. 2011). However, researches on the influences of TiO_2 NP interaction with organic pollutants are limited and urgently needed. In a study, Shi et al. (2010) tested the exposure toxicities of p,p′-DDT and TiO_2 NPs at low concentrations on L-02 cells of human hepatocytes. The combination of p,p′-DDT and TiO_2 NPs induced higher toxicity than TiO_2 NPs or p,p′-DDT alone and synergistically enhanced genotoxicity, increase in oxidative stress, oxidative DNA adducts, DNA breaks, and chromosomal damage in L-02 cells. In another study conducted by Canesia et al. (2014), both synergistic and antagonistic effects of TiO_2 NPs in combination with 2,3,7,8-tetrachlorodibenzo-p-dioxins

(2,3,7,8-TCDD), depending on the type of cell/tissue, and the biomarker response on the marine bivalve (*Mytilus galloprovincialis*) were reported. In this way, we found more severe damages in the gill and intestine organs following exposure to the combination of diazinon and TiO_2 NPs than those found at TiO_2 NPs and diazinon groups separately. Moreover, the results of statistical analysis indicated also notable significant differences in gill parameters of common carp when exposed to mixed diazinon and TiO_2 NPs. These results suggest that the toxic effects of diazinon in common carp in the joint presence of TiO_2 NPs were synergistic effect. The mechanism of a toxic effect of diazinon is an inhibition of a whole series of enzymes and mainly of inhibition of acetylcholinesterase (AChE) in the central and peripheral nervous system (Mahboob et al. 2011). Toxicities of diazinon alone have been well documented; however, comprehensive information from the mechanism of toxicity of diazinon in the presence of other materials, including nanoparticles on organs of aquatic organisms, have not been reported, so further research is needed in this area. Based on the findings of this research, the toxicity of diazinon caused several histopathological anomalies on the tissues of common carp and the damages were increased in the presence of titanium dioxide nanoparticles. In conclusion, it appears that the joint presence of TiO_2 NPs can potentially increase the effects of diazinon on the tissues of carp.

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Compliance with ethical standards All procedures involving the experimental use of animals were approved by the Animal Ethics Committee (MUK.REC.1395–55), a branch of the Research Council of the Ethics Committee in Kurdistan University of Medical Sciences, Iran, and administered by the National Animal Ethics Advisory Committee.

Conflict of interest The authors declare that they have no conflict of interest.

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