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Histopathological effects following short-term coexposure of *Cyprinus carpio* to nanoparticles of TiO_2 and CuO

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Abstract The aim of this research was to investigate the coexposure of nanoparticles of titanium dioxide (TiO_2) and copper oxide (CuO) on the alterations of the gill, intestine, kidney, and liver tissues of carps (*Cyprinus carpio*). In this study, carps (length 23 ± 1.5 cm; weight 13 ± 1.3 g) were divided into six groups of 15 each and exposed to 2.5 and 5.0 mg L^{-1} of CuO nanoparticles (NPs), 10.0 mg L^{-1} of TiO_2 NPs, and 2.5 and 5.0 mg L^{-1} of CuO NPs + 10.0 mg L^{-1} of TiO_2 NP mixture. Fish were sampled for histopathological studies after hematoxylin-eosin staining. Results indicated that the more kinds of histopathology anomalies observed with CuO NP and TiO_2 NP mixture were broadly of the same type as CuO NPs and TiO_2 NPs alone, but the severity or incidence of injuries of gill, intestine, liver, and kidney of carps in the mixture of CuO NPs + TiO_2 NPs was higher than that of each NP alone. Moreover, behavioral changes in carps exposed to CuO NP and TiO_2 NP mixture such as hyperactivity, loss of balance, and convulsions were higher than those to CuO NPs and TiO_2 NPs alone. In conclusion, the presence of TiO_2 NPs enhanced the effects of NPs of copper oxide in terms of histopathological changes in carps.

Keywords Histopathology · Coexposure · CuO · TiO_2 · Nanoparticles · Acute toxicity

Introduction

Nanoparticles (NPs) are applied in a variety of consumer products at large such as fillers, opacifiers, semiconductors, catalysts, cosmetics, and microelectronics (Shahmoradi et al. 2011; Maleki and Shahmoradi 2012). Titanium dioxide (TiO_2) is the naturally occurring oxide or mixed oxide with other metals and includes three crystalline forms: anatase, brookite, and rutile (Clemente et al. 2012). TiO_2 NPs have been used in various sectors such as degrading organic contaminants and germs, UV-resistant material, cosmetic products, antiseptic and antibacterial compositions, and paper industry. Moreover, copper oxide nanoparticles (CuO NPs) are widely applied in numerous fields such as antifouling paints, bactericides, batteries, electronics, gas sensors, and textiles (Chowdhuri et al. 2004; Zhang et al. 2005; Almeida et al. 2007; Buffet et al. 2011; Song et al. 2015). Thus, large quantities of these NPs may be

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released into the aquatic environment and may cause adverse effects on aquatic organisms.

The release of metal oxide NPs in environment is a worldwide concern due to their bioaccumulation, toxicity, and presence in consumer products and industrial pollutants (Melegaria et al. 2013). The acute and chronic toxicity effects of NPs of TiO_2 and CuO on the organs of aquatic organisms such as fish have been illustrated by many researchers (Federici et al. 2007; Speisky et al. 2009; Khabbazi et al. 2015). According to previous articles by different scholars, a series of injuries such as reactive oxygen species-derived oxidative stress (Gomes et al. 2011; Buffet et al. 2011), DNA damage, and biochemical lesions (Karlsson et al. 2008; Abdel-Khalek et al. 2015) were reported in the exposure to the CuO NPs of human and aquatic organisms, which may suggest ecological implications of CuO NP toxicity for human and other organisms. Adama et al. (2015) demonstrated that the increasing exposure to CuO NPs causes reduction in the reproduction and length and enhances copper uptake in *Daphnia magna*. However, the information about ecotoxicological risk of coexisting NPs and other pollutants in the organism's organs such as fish is limited and, in some cases, even nonexistent.

Nanomaterial behavior and toxicity in the presence of other nanomaterials and environmental contaminants could be changed. Several parameters affect the toxicity of nanomaterials, including nanomaterial type, size, and concentration, as well as exposure time, species, and environmental conditions. In recent years, several investigations have revealed the coexposure of NPs with other environmental contaminants such as TiO_2 NPs and Cd on carps (Zhang et al. 2007), TiO_2 NPs on toxicity of Ag NPs (Zou et al. 2014), and cadmium telluride quantum dots (CdTe QDs) on toxicity of Cu on zebrafish (Zhang et al. 2013). These findings reveal that manufactured NPs may have important direct effects and indirect impacts on aquatic species such as fish by interactions with other environmental contaminants. However, to assess the toxicity of coexistence of NPs with other environmental pollutants, further comprehensive studies are needed for a proper understanding of the potential risks of NPs. Therefore, the aim of the present research was to investigate the coexistence effects of NPs of TiO_2 and CuO on the gill, intestine, kidney, and liver alterations of common carps, *Cyprinus carpio*.

Materials and methods

NPs and characterizations

TiO_2 NPs (anatase/rutile, 99+ (% and CuO NPs (99+ %) used in this study were produced by US Research Nanomaterials Inc. (3302 Twig Leaf lane, Houston, TX77084) and purchased from Nanosany Co. (Mashhad, Iran). Complete characterization of both nanomaterials was provided by Nanosany Co. Briefly, morphology and mean unaggregated particle diameters of NPs of TiO_2 and CuO were determined by scanning electron micrographs (SEM) and transmission electron microscopy (TEM), and for the analysis of phase structure of both NPs, X-ray diffraction (XRD) was used (Fig. 1). Dynamic light scattering (DLS; Zetasizer Nano (ZS) model ZEN3600, Malvern Instruments Ltd., Worcestershire, UK) was applied to determine the zeta potential and hydrodynamic diameter of NPs of CuO and TiO_2 in deionized water.

Test organism and experimental condition

Common carps (*C. carpio*; length 23 ± 1.5 cm; weight 13 ± 1.3 g) were bought from a local aquaculture farm in Gilan province, Iran, and were acclimated in 1000-L tanks for 1 month and maintained in aquariums under a 12-h light/dark. During the acclimatization period, the carps were fed fish food every day and were deprived of food 1 day prior to starting the experiments. Water used to study toxicity in this research has the characteristics of 5° dGH for hardness, 600 ± 10 μS cm^{-1} for conductivity, 7.5 ± 0.5 for pH, 6.0 ± 0.6 mg L^{-1} for dissolved oxygen (DO) content, and 26.0 ± 1 °C for temperature.

Acute toxicity

According to results of Linhua et al. (2009) and Lee et al. (2012), one sublethal concentration of NPs of TiO_2 (10 mg L^{-1}) was selected. Moreover, to determine the appreciated concentrations of CuO NPs for acute experiments, a series of toxicity experiments were carried out based on OECD 203 (OECD 1992). Thereafter, for certain tests, fish were treated with 15, 20, 25, 30, 35, 40, 45, 50, and 55 mg L^{-1} of NPs for 96 h. During the testing period, the behavior and mortality of the carps were recorded, and dead fish, after being recorded, were immediately removed from the aquariums tested. Lethal concentrations at 50 % (LC_{50}) and confidence interval values were calculated by

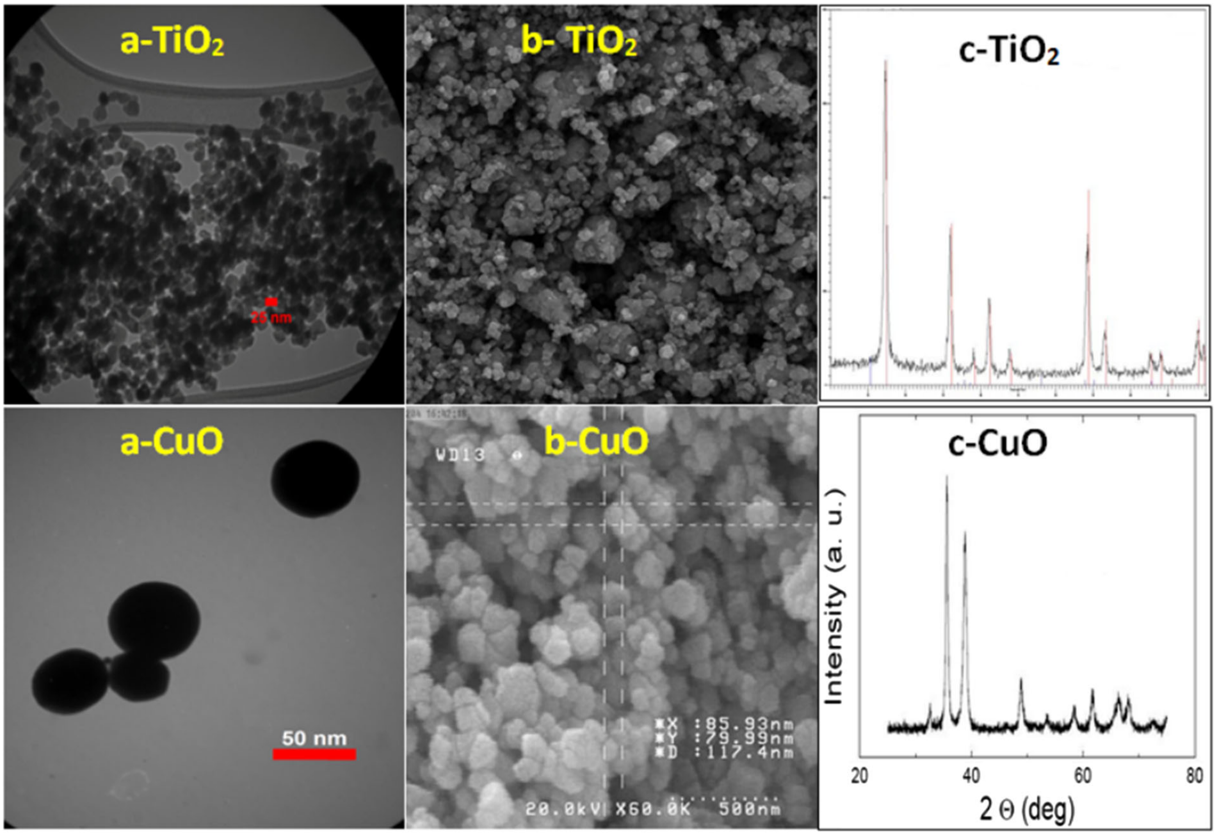

Fig. 1 TEM (**a**), SEM (**b**), and X-ray (**c**) images of tested TiO_2 NPs and CuO NPs

means of the Probit Analysis Program, version 1.5 (US Environmental Protection Agency). Moreover, if the calculated chi-square test statistic for heterogeneity (2.49) was lower than the tabular value (16.91), all results were accepted. As described by Kumar et al. (2007), the behavior pattern of carps was recorded.

After determining the 96-h LC_{50} for CuO NPs, two sublethal concentrations of CuO NPs (including 2.5 and 5.0 mg L^{-1}), which were approximately equal to 1/20th and 1/10th of LC_{50} concentrations, were selected to study their toxicity alone or in combination with 10 mg L^{-1} TiO_2 NPs. Briefly, the fish were divided into six groups of 15 each in 55-L glass aquariums, exposed to different treatments in triplicate and for 4 days: the first group was the control (without adding any chemicals), while the second group was those that are exposed to TiO_2 NPs (10 mg L^{-1}), the third and fourth groups to CuO NPs (2.5 and 5.0 mg L^{-1}), and the fifth and sixth groups to mixtures of TiO_2 NPs (10 mg L^{-1}) and CuO NPs (2.5 mg L^{-1}), and TiO_2 NPs (10 mg L^{-1}) and CuO NPs (5.0 mg L^{-1}), respectively. In order to minimize decreases in the CuO and TiO_2 concentrations during the experiments, 50 % of the water of each aquarium was renewed every day.

Histological examinations

Histological observations were performed by hematoxylin-eosin staining. Following 4-day exposure to different groups of NPs, the carps were carefully anesthetized. Tissues of gill, intestine, kidney, and liver of carps were dehydrated in graded ethanol series and embedded in paraffin. After that, sections with a thickness of 5 μm were prepared by a microtome (MicroTec, Rotary microtome, CUT 4050). These slides were observed microscopically (Nikon Eclipse- E200; Mansouri et al. 2015). Histopathological anomalies in the carp tissues were categorized as none (−), mild (+), moderate (++), and severe (+++) effects. In this study, experimental procedures conformed to the Ethics Committee of the Kurdistan University of Medical Sciences (MUK.REC.1393.198). Compliance with ethical standards. In this study, experimental procedures conformed

Table 1 Characteristics of nanoparticles of TiO_2 and CuO and their mixture determined by DLS

Groups	Zeta potential (mV)	Polydispersity index (PDI)	Hydrodynamic diameter (nm)
TiO_2 NPs (10 mg L^{-1})	−14.18	0.5	22.2
CuO NPs (2.5 mg L^{-1})	−5.07	0.2	26.6
CuO NPs (5.0 mg L^{-1})	−1.46	0.08	35.0
CuO NPs (2.5 mg L^{-1}) and TiO_2 NPs	−21.5	0.4	42.7
CuO NPs (5.0 mg L^{-1}) and TiO_2 NPs	−4.66	0.4	61.5

to the Ethics Committee of the Kurdistan University of Medical Sciences (MUK.REC.1393.198).

Results

NPs and characterizations

The main characteristics of TiO_2 NPs in this research were assessed including bulk density (0.46 g cm^{-3}), loss of weight on ignition (0.99 %), loss of weight in drying (0.48 %), pH (5.5–6.0), and specific surface area (20 m^2 g^{-1}). Also, other characteristics of CuO NPs were 20 m^2 g^{-1} for specific surface area (SSA), 6.4 g cm^{-3} for true density, and 0.79 g cm^{-3} for bulk density. According to the results of the dynamic light scattering (DLS) method in Table 1, the hydrodynamic diameters of the TiO_2 NPs, CuO NPs (2.5 mg L^{-1}), CuO NPs (5.0 mg L^{-1}), and mixtures of TiO_2 NPs and CuO NPs (2.5 mg L^{-1}) and TiO_2 NPs and CuO NPs (5.0 mg L^{-1}) were 26.9, 26.68, 35.07, 55.17, and 42.7 nm, respectively. Results illustrated that the presence of TiO_2 NPs enhanced the zeta potential, polydispersity index, and hydrodynamic diameter of CuO NPs in comparison with the CuO NPs alone.

Table 2 Results of acute toxicity (LC_{50}) of nanoparticles of CuO (mg L^{-1}) calculated by EPA method

Exposure point	Concentration	Lower	Upper
LC1.0	10.2	6.1	14.1
LC5.0	16.3	11.1	20.5
LC10.0	20.8	15.5	25.1
LC15.0	24.6	19.2	28.9
LC50.0	49.6	43.8	57.5
LC85.0	100.11	81.1	140.9
LC90.0	118.1	92.8	175.9
LC95.0	151.1	113.2	244.7
LC99.0	239.7	163.9	456.3

Acute toxicity test

The results of the acute toxicity of CuO NPs in common carps are shown in Table 2. Accordingly, the 96-h median lethal concentration of CuO NPs was 49.6 mg L^{-1}. No mortality was observed during the experimental period in controls. Moreover, the intercept and slope of acute toxicity of CuO NPs were −0.7687 and 3.4017, respectively (Table 3). Hence, the further studies such as histopathological studies were analyzed below the concentration of LC_{50} value. Common carps exposed to CuO NPs and TiO_2 NPs exhibited abnormal behaviors such as enhanced rate of opercular activity, rapid swimming, convulsions, hyperactivity, loss of balance, and increased surfacing activity with the changing groups of TiO_2 NPs and CuO NPs in comparison with the control group (Table 4). Exposure to NPs led to lethargy and mucus secretion from the body. Moreover, fish that swam to the surface in the different experimental groups were higher than those in the control group, while behavioral changes and mortality were not observed in the control group. In addition, abnormal behavior of common carps exposed to CuO NP and TiO_2 NP mixture was higher than that exposed to CuO NPs and TiO_2 NPs alone.

Table 3 Characteristics of acute toxicity of CuO NPs estimated by EPA method

Parameters	Estimate	Std. err.	95 % Confidence limit
Intercept	−0.768763	0.758475	(−2.255375, 0.717849)
Slope	3.401746	0.462183	(2.495867, 4.307625)

Table 4 Impact of CuO NPs and TiO_2 NPs on the behavior of common carps for 96 h

Parameters	CuO NPs[a] and TiO_2 NPs	CuO NPs[b] and TiO_2 NPs	CuO NPs[a]	CuO NPs[b]	TiO_2 NPs	Control
Convulsions	+++	+++	+	+	−	−
Loss of balance	+++	++	+	+	−	−
Rate of opercular activity	++	++	+	+	+	−
Rate of swimming	++	++	+	++	+	+
Hyperactivity	+++	++	++	+	+	−

(−) None, (+) mild, (++) moderate, and (+++) strong

[a] *HC* high concentration (1/10th; 5.0 mg L^{-1})

[b] *LC* low concentration (1/20th; 2.5 mg L^{-1})

Gill and intestine histopathology

The gill and intestine histopathology anomalies of common carps in acute toxicity period are shown in Fig. 2. According to results, the gills and intestine of control fish indicated only some small histopathological anomalies, while exposure to different groups of NPs has made greater intensity of tissue damages of dilated and clubbed tips, mucus secretion, edema (Oe), hyperplasia (Hp), lamellar fusion (F), synechiae of lamellae, epithelium shortening (ES), aneurism (An), and necrosis. Lamellar fusion and hyperplasia anomalies in secondary lamellae were recognized on filaments of the gill in half of carps from mixture of CuO and TiO_2 NP treatment. Moreover, fusion and hyperplasia are the highest and severe damages found in the gill of the carps.

According to our results, the most important alterations in intestine tissues observed include degeneration, integration of villi, expansion at villi structure, increase in the number of blood cells, and necrosis and erosion. The intestine and gill tissues of carps indicated different histological anomalies depending on the groups. The histopathological results in Table 5 indicated that the intensity of anomalies of gill tissue, such as lamellar fusion, edema, hyperplasia, epithelium shortening, and aneurism, in the mixture of CuO NPs and TiO_2 NPs was higher than that in NPs alone. Moreover, the intensity of intestine anomalies such as degeneration (D) and the increase in the number of blood cells (INBC) in the mixture of CuO NPs and TiO_2 NPs were higher than those in NPs alone. In addition, the gill and intestine histopathology anomalies of common carps exposed to the CuO NPs in the presence of TiO_2 NPs were increased.

Liver and kidney histopathology

The histological anomalies in the kidney and liver of carps due to toxicity effects caused by NPs of TiO_2 and CuO are illustrated in Fig. 3. In control, no significant recognizable changes were observed in kidney and liver tissues during the experimental period. Unlike the control group, various histopathological anomalies were identified in the kidney and livers of carps exposed to the different groups of NPs. The intensity of some of histopathological alterations such as hepatocyte necrosis (HN), hemorrhage (H), dilated sinusoids (DS), blood sinusoids (BS), and melanomacrophage aggregates (MA) in the liver of carps exposed to NPs of TiO_2 and CuO mixture was much higher compared to those fish exposed to TiO_2 and CuO NPs alone as well as control group (Table 6). Moreover, increased Bowman's space, renal tubule degeneration, glomerular necrosis, necrosis of hematopoietic tissue, and congested blood vessels were the most important alterations in kidney tissues of common carps. In addition, according to our study, the effects of NPs of CuO on the histological anomalies of the kidney and liver of common carps in the joint presence of TiO_2 NPs were increased.

Discussion

Acute toxicity

The present study indicated that the 96-h LC_{50} of CuO NPs for common carps (*C. carpio*) was 49.6 mg L^{-1}, and according to the toxicity classification of chemical materials (USEPA 2010), it is suggested that CuO NPs in freshwater be classified as slightly toxic substances. Griffitt et al. (2007) reported that the LC_{50} concentration

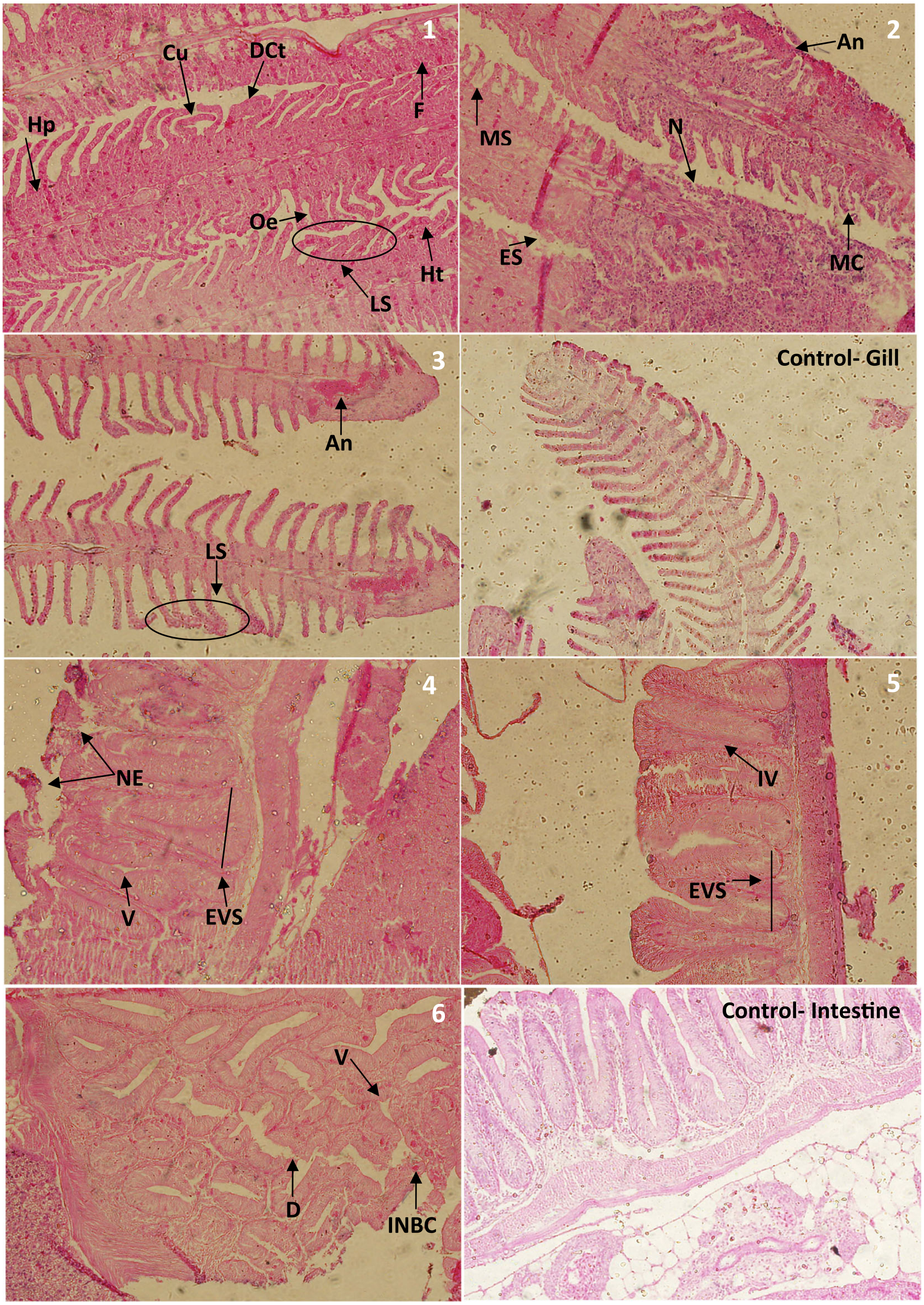
1
Cu
DCt
F
Hp
Oe
Ht
LS
2
An
MS
N
ES
MC
3
An
LS
Control- Gill
4
NE
V
EVS
5
IV
EVS
6
V
D
INBC
Control- Intestine

Fig. 2 Gill and intestine morphology in common carps in acute period (4 days). The gills and intestines of control fish illustrated only some small histopathological anomalies, while all treatments in gill organs (*1–3*) indicated anomalies such as curvature (*Cu*), dilated and clubbed tips (*DCt*), edema (*Oe*), hyperplasia (*Hp*), mucus secretion (*Ms*), lamellar fusion (*F*), aneurism (*An*), hypertrophy (*Ht*), lamellar synechiae (*LS*), epithelium shortening (*ES*), dilated marginal channel (*MC*), and necrosis (*N*) and, in intestine organs (*4–6*), injuries that include degeneration (*D*), integration of villi (*IV*), increase in the number of blood cells (*INBC*), vacuolation (*V*), expansion at villi structure (*EVS*), necrosis, and erosion (*NE*)

of Cu NPs was 1.5 mg L^{-1} during 48 h and the value obtained is toxic to zebrafish (*Danio rerio*). Moreover, Jahanbakhshi et al. (2015) recorded that 96-h LC_{50} of nano-CuO was 2.19 mg L^{-1} in roach (*Rutilus rutilus*), explaining that the LC_{50} values showed that CuO NPs were moderately toxic to the organisms, while Abdel-Khalek et al. (2015) illustrated that the 96-h LC_{50} of CuO NPs was 150 mg L^{-1} in Nile tilapia (*Oreochromis niloticus*). Acute toxicity of copper forms on fish depends on different parameters, but CuO potential toxicity should not be ignored. According to this research, some disorders in behavioral patterns of fish such as mucous secretion and lethargy from the body of common carps were observed. The behavioral changes observed in this research were consistent with those in the previous results on other NPs (Perera and Pathiratne 2012) and metals (Begum et al. 2006; Mishra and Mohanty 2008).

Gill histopathology

The gill tissue as vital organs in the body of fish plays several critical roles such as osmoregulation, respiratory gas exchange, and body fluid permeability balance; moreover, this tissue, which has a large superficial area of the epithelium and a direct contact between organ and water, is more susceptible to chemical pollutant effects in aquatic systems (Baramaki et al. 2012; Nowrouzi et al. 2012; Maleki et al. 2015). According to our observations in this research, the major gill responses of carps exposed to CuO NPs, TiO_2 NPs, and CuO NP + TiO_2 NP mixtures were aneurism, fusion, gill epithelial hyperplasia, and lamellar synechiae, which might cause respiratory and osmoregulatory disorders. These tissue damages, moreover, play a role in defense mechanisms against pollutants as well as in preventing further damage of toxic substances. The formation of aneurism was observed in common carp gills exposed to different groups of NPs, while the extent and severity of this lesion were higher in CuO NP + TiO_2 NP mixtures than those in CuO NPs and TiO_2 NPs alone. Aneurism is the blood-filled bulge in the

Table 5 Histopathological anomalies on the gill and intestine organs of carps exposed to treatment groups of NPs and the control group

Organs/groups	Anomalies								
Gill	Cu	Oe	Hp	LS	F	DCt	ES	An	N
CuO NPs[a] and TiO_2 NPs	++	++	+++	++	+++	+	++	++	++
CuO NPs[b] and TiO_2 NPs	+	+	++	++	++	+	+	++	+
CuO NPs[a]	−	+	++	+	+	+	−	−	−
CuO NPs[b]	−	+	++	+	+	+	−	+	−
TiO_2 NPs	+	+	+	−	+	+	−	−	−
Control	+	−	−	−	−	−	−	−	−
Intestine	D	NE	INBC	IV	EVS	V			
CuO NPs[a] and TiO_2 NPs	+++	++	++	++	++	++			
CuO NPs[b] and TiO_2 NPs	++	++	+	+	++	++			
CuO NPs[a]	++	+	−	+	+	+			
CuO NPs[b]	+	+	−	−	−	+			
TiO_2 NPs	+	−	−	+	−	+			
Control	−	−	−	+	−	+			

None (−), mild (+), moderate (++), and severe (+++)

[a] *HC* high concentration (1/10th; 5.0 mg L^{-1})

[b] *LC* low concentration (1/20th; 2.5 mg L^{-1})

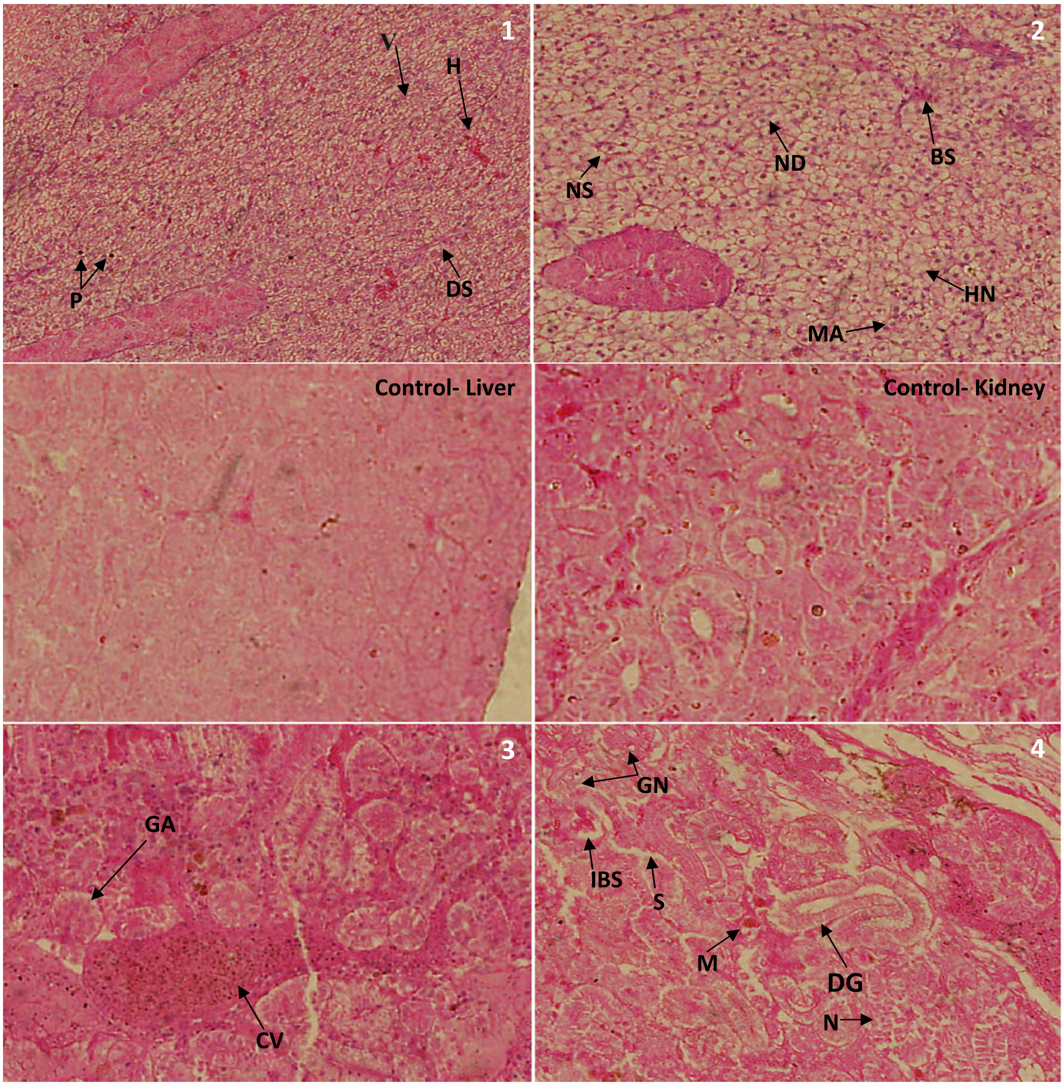

Fig. 3 Liver and kidney morphology in common carps in acute period (4 days). The liver and kidney of control fish indicated only some small histopathological alterations, while all treatments indicated anomalies such as vacuolization (*V*), hepatocyte necrosis (*HN*), nuclear degeneration (*ND*), hemorrhage (*H*), dilated sinusoids (*DS*), narrowing of sinusoids (*NS*), blood sinusoids (*BS*), pigmentation (*p*), melanomacrophage aggregates (*MA*) in liver tissue (*1* and *2*), glomerular alteration (*GA*), increased Bowman's space (*IBS*), melanomacrophages (*M*), renal tubule degeneration (*Dg*), enlarged sinusoids (*S*), glomerular necrosis (*GN*), necrosis of hematopoietic tissue (*N*), and congested blood vessel (*CV*) in kidney tissue (*3* and *4*)

wall of a blood vessel, and the breakdown of vascular integrity may cause some damages in fish such as (1) disturbances in blood flow in the gills, (2) risk of rupture, and (3) bleeding or death (Stentiford et al. 2003; Flores-Lopes and Thomaz 2011). Al-Bairuty et al. (2013), in a similar research, illustrated that the nano-Cu caused a high rate of aneurism in the branchial vasculature of rainbow trout (*Oncorhynchus mykiss*). In experimental studies, Rajkumar et al. (2015) and Mansouri et al. (2015) observed lamellar aneurisms in the gills of *Labeo rohita* and *D. rerio* exposed to Ag NPs and Co NPs, respectively. In another study, Stentiford et al. (2003) reported severity of this lesion in fish from contaminated areas and confirmed that it could be related to presence of pollutants in the water.

Lamellar synechiae was observed as one of alterations in the gill of common carps exposed to different groups of NPs, and the results of this study similar to the results of Nero et al. (2006) illustrated that the chloride cell proliferation and epithelial cell proliferation led to synechiae in

Table 6 Histopathological anomalies on the tissues of liver and kidney of carps exposed to treatment groups of NPs and the control group

Organs/groups	Anomalies						
Liver	V	HN	ND	H	DS	BS	MA
CuO NPs[a] and TiO_2 NPs	++	++	++	++	++	+++	+++
CuO NPs[b] and TiO_2 NPs	++	++	++	++	+	++	+++
CuO NPs[a]	+	+	++	+	+	+	++
CuO NPs[b]	+	+	+	+	+	+	+
TiO_2 NPs	+	−	+	+	−	+	+
Control	+[a]	−	−	−	−	+	−
Kidney	GA	IBS	GN	N	CV	S	
CuO NPs[a] and TiO_2 NPs	+++	++	+++	+++	+++	+++	
CuO NPs[b] and TiO_2 NPs	+++	++	++	+++	+++	++	
CuO NPs[a]	++	+	+	+	+	+	
CuO NPs[b]	+	+	+	+	+	+	
TiO_2 NPs	+	+	−	+	−	+	
Control	+	+	−	−	−	−	

None (−), mild (+), moderate (++), and severe (+++)

[a] HC: high concentration (1/10th; 5.0 mg L^{-1})

[b] LC: low concentration (1/20th; 2.5 mg L^{-1})

gill lamellae. Also, gill clubbing is due to excess mucus production. Exposure to pollutants such as NPs increased mucus secretion from the epithelium of the secondary lamellae, and this condition resulted in the fusion of secondary gill lamellae, resulting in impaired respiration (Mansouri et al. 2015). Given the results of the present study, lamellar fusion was one of the most prominent damages observed microscopically. It often resulted from epithelial hypertrophy and hyperplasia, and this alteration may be an indication of their either reaction to toxicant such as NP intake or adaptation to prevent pollutant entry through the gill surface (Cerqueira and Fernandes 2002; Olurin et al. 2006). A similar alteration has been reported by several authors following acute or chronic intoxication of fish by various NPs such as TiO_2 NPs (Hao et al. 2009), Co NPs (Mansouri et al. 2015), and Ag NPs (Mansouri et al. 2015).

Intestine histopathology

Intestine is one of the target organs coming into contact with food-borne aquatic pollutants and is very much vulnerable to ingested toxic substances. In this study, most important anomalies in intestine organs exposed to different groups of NPs included degeneration, expansion at villi structure, increasing number of blood cells, and necrosis and erosion. Although the damages caused by the CuO NPs and Cu NP + TiO_2 NP mixture were somewhat similar, the severity of damage caused by Cu NP + TiO_2 NP mixture was higher than that by the CuO NPs alone, and the quantitative analysis confirmed this difference in severity. Studies have shown that intestinal microvilli make a large surface area for absorption of substances and the increase in length or density of intestinal microvilli can enhance absorptive ability by intestine (Sang and Fotedar 2010; Daniels et al. 2010). Results indicated that TiO_2 NPs + CuO NP mixture could increase intestinal microvilli length of carps, which suggested that TiO_2 NPs could enhance the toxicity of CuO NPs on the intestinal microvilli. Federici et al. (2007) revealed that the nano-TiO_2 caused the erosion and fusion in villus and vacuolation of the intestinal epithelial cells in rainbow trout (*O. mykiss*). In a similar research using rainbow trout (*O. mykiss*), after 21 days of exposure to sublethal concentrations of Ag NPs, inflammation and necrosis in the intestinal tissues were reported by Johari et al. (2015).

Liver histopathology

Histological anomalies in the liver of common carps include vacuolization, HN, nuclear degeneration, DS, BS, pigmentation, and MA. Similar changes were observed in the liver of common carps (*C. carpio*) exposed to TiO_2 NPs (Hao et al. 2009), rainbow trout (*O. mykiss*) exposed to Cu NPs (Al-Bairuty et al. 2013), and Siberian sturgeon (*Acipenser baerii*) exposed to Cu NPs (Ostaszewska et al. 2015). In another study by Smith et al. (2007), it was shown that rainbow trout (*O. mykiss*) exposure to single-walled carbon nanotubes (SWCNT) caused similar types of histopathology alterations in the liver structure. As liver is one of the most important tissues of detoxification and active metabolism, histological anomalies in this tissue have been used as suitable biomarkers for assessing the health of fish exposed to different NPs (Al-Bairuty et al. 2013; Jayaseelan et al. 2014). According our results, HN and nuclear degeneration were the most important histopathological effects observed in the liver tissue samples. Histological anomalies such as degeneration and necrosis of hepatocytes in the liver tissues may be due to the effects of NPs accumulation and increase in their concentration over time (Mohamed 2001).

Kidney histopathology

Most lesions of the kidney in this study included alterations in Bowman's space, renal tubule degeneration, melanomacrophages, glomerular necrosis, and necrosis of hematopoietic tissue. Kaya et al. (2016), in a similar research, reported that the ZnO NPs lead to alterations in Bowman's space, degeneration of renal tubule, vacuolation, MA, and necrosis of hematopoietic tissue in the kidney of tilapia (*Oreochromis niloticus*). Based on the results of this research, CuO NP and TiO_2 NP mixture caused increase in the intensity of lesions in the kidney and liver compared to TiO_2 NP and CuO NP groups. Overall, the presence of TiO_2 NPs leads to an enhance in the toxicity of NPs of CuO and the synergistic effect of the toxicity of these NPs leads to increases in the intensity of severity of common carp organs. Some studies have illustrated that the coexposure of NPs with other NPs and environmental pollutants have the ability to reduce their toxicity effects on the body of aquatic organisms (Zou et al. 2014; Mansouri et al. 2016), whereas other investigations have reported that the presence of NPs increases the toxicity of other NPs and environmental pollutants (Zhang et al. 2007; Shi et al. 2015). Rosenfeldt et al. (2015) illustrated that the presence of TiO_2 NPs decreased the toxicity effects of copper on the benthic amphipod *Gammarus fossarum*, while the results of Canesi et al. (2014) presented that both the synergistic and antagonistic effects of TiO_2 NPs in combination with TCDD on the marine bivalve depend on several parameters such as biomarker response and cell and tissue types. More studies are needed to evaluate the behavior of NP coexistence with other pollutants to understand the potential risks of environmental pollution by NPs.

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Compliance with ethical standards In this study, experimental procedures conformed to the Ethics Committee of the Kurdistan University of Medical Sciences (MUK.REC.1393.198).

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