Comparative toxicity of nanoparticulate and ionic copper following dietary exposure to common carp (Cyprinus carpio)

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ABSTRACT

Copper is an essential element for the normal growth and survival of all organisms including fish. However, its excessive presence in the environment can cause bioaccumulation and aquatic toxicity. The aim of the present study was to compare the dietary toxicity effects of two different Cu compounds, copper oxide nanoparticles (CuO-NPs) and ionic copper (CuSO₄) in juvenile common carp, Cyprinus carpio. To prepare experimental diets, two nominal concentrations of 100 and 1000 mg Cu kg\textsuperscript{-1} diet were added to a basal diet. Carp (n = 450, average initial weight of 35.94 ± 5.35 g) were fed on the Cu-supplemented diets and basal diets for two 21-day courses as dietary exposure and recovery periods, respectively. The growth performance, survival rate and blood biochemical indices as well as copper accumulation in target organs of fish were investigated at the end of each exposure period. The results showed that the weight gain (WG) of carp significantly decreased coincident with increasing concentration of the both dietary Cu forms (P = 0.00). Both Cu sources at concentrations of 100 mg kg\textsuperscript{-1} diet decreased the survival rate of fish (P = 0.003), likely due to more feed intake and thus increased copper toxicity. The both forms of dietary Cu at two different concentrations significantly decreased the plasma glutamate oxaloacetate transaminase (GOT) level compared to the control group (P = 0.008). Fish exposed to diets containing Cu sources except 100 mg Cu kg\textsuperscript{-1} of CuO-NPs showed the lower glutamate pyruvate transaminase (GPT) activity in comparison to the control (P = 0.00). The plasma sodium level increased in the 1000 mg CuO-NPs kg\textsuperscript{-1} diet was significantly lower than the control (P = 0.001). The plasma potassium level increased in the all Cu-supplemented groups except 100 mg kg\textsuperscript{-1} of CuO-NPs after the dietary exposure period (P = 0.035). The copper accumulation was dose-dependent in all target organs. In 100 mg Cu kg\textsuperscript{-1} dietary groups, the highest Cu content was observed in the intestine (P = 0.00). The results demonstrated the enhanced toxicological responses in fish after 21 days of dietary exposure, but the levels of most of biochemical indices and tissues Cu content decreased or returned to the control values after the recovery period.

1. Introduction

Copper (Cu) as an essential trace element plays an important role in normal metabolic functioning of organisms (Watanabe et al., 1997). It acts in hemoglobin synthesis, hematopoiesis, cellular respiration, bone formation and as a cofactor for numerous enzymes like super oxide dismutase, cytochrome c oxidases, ferroxidase, lysyl oxidase, monooxygenases and ceruloplasmin (Isani et al., 2013; Lorentzen et al., 1998). In aquaculture, Cu is used in the soluble forms of CuSO₄ as an essential trace element in diets as well as a therapeutic chemical for controlling algal bloom and bacterial infections (Griffin and Mitchell, 2007; Isani et al., 2013). Dietary copper requirement of 3–10 mg Cu kg\textsuperscript{-1} dry diet has been reported for teleost fish (Clearwater et al., 2002). In the last few decades, contamination of aquatic environments by Cu has been increased likely due to excessive use of Cu-based products (IPCS, 1993; Mustafa et al., 2012). Furthermore, the transfer of metals through the food chain and dietary uptake of metals are the main causes of long-term contamination in wild fish (Dallinger et al., 1987; Yi et al., 2011). Rainbow (2007) suggested that concentration of copper in natural food (invertebrates) could increase up to 3750 mg kg\textsuperscript{-1} in polluted areas. Fish can accumulate Cu in intestine, liver, kidney and gill tissues depending on the source and exposure...
route (Giles, 1984; Sorensen, 1991). Accumulation of Cu in excess of cellular needs may damage cells and alter their physiological functions (Gottschalk et al., 2009; Nussey et al., 1995). Copper can exert its negative effect on fish through reduced growth, reproductive problems, ionoregulation impairments and oxidative stress via the creation of reactive oxygen species (ROS) (Ajani and Akpolih, 2010; Bebiani et al., 2004; Couture et al., 2008; McGeer et al., 2000). Shaw and Handy (2006) showed that dietary Cu level of about 1500 mg kg\(^{-1}\) food decreased growth in Nile tilapia (Oreochromis niloticus) after feeding for 42 days. Due to the importance of fish as a food resource for humans and as a major ecosystem component, the assessment of bioaccumulation pattern of metals in fish organs is essential (Kim and Kang, 2004).

Today, nanotechnology science is growing rapidly and subsequently the manufacture and use of manufactured nanomaterials (MNMs) has been increased (Wan et al., 2018; Vance et al., 2015). Copper oxide nanoparticles (CuO-NPs) because of their unique physico-chemical properties are commonly used in many consumer products and industrial technologies (Montes et al., 2012; Phiwdang et al., 2013). It is inevitable that large-scale production and consumption of MNMs will result in their discharge to aquatic ecosystems, which causes hazardous impacts on aquatic animals (Fabrega et al., 2011; Peralta-Vide et al., 2011; Krzyzewska et al., 2016). Exposure routes may affect the uptake, bioaccumulation and toxicity of CuO-NPs in aquatic animals. Some studies have reported the transfer and bioaccumulation of CuO-NPs in aquatic food chain. Ates et al. (2014) reported the transfer of CuO-NPs from Artemia salina to goldfish (Carassius auratus). Nemati et al. (2019) also found that copper oxide nanoparticles could be absorbed by Artemia salina and transfer from nauplii of Artemia to cichlid (Amatitlania nigrofasciata) larvae. Recently, CuO-NPs are used as a novel Cu source in feed supplements (Ates et al., 2014; Majewski et al., 2017; Wang et al., 2018). In Wang et al. (2018), the growth, tissue Cu burden and immune responses of Russian sturgeon Acipenser gueldenstaedtii fed on diets supplemented with 4 mg Cu kg\(^{-1}\) in nano CuO form were higher than fish fed the same Cu content in the CuSO\(_4\) form. Enrichment of red sea bream, P指导us major diet with copper NPs also enhanced growth and health of fish (El-Basini et al., 2016a, 2016b). Despite the positive effects of dietary copper NPs, the excessive accumulation of these particles in organs such as gills and liver of fish may cause genotoxicity at cellular level (Song et al., 2012), oxidative stress, disruption of normal ionoregulatory homeostasis (Handy, 2003) and necrosis of tissues Al-Bairuty et al., 2013(Al-Bairuty et al., 2013). Biochemical constituents of the body had important role in the body’s construction and energy metabolism (Siddiqui and Noorjahan, 2018). Therefore, assessment of biochemical indices in blood and tissues could help to identify the general health status of animals as well as target organs of toxicity and the physiological stress response of fish (David et al., 2010; Dawood et al., 2016). El-Basini et al. (2016a, 2016b) examined the effects of dietary copper (bulk & nano) particles on blood biochemical profiles including glucose level, glutamyl oxaloacetic transaminase (GOT) and glutamic-pyruvate transaminase (GTP) activities of Red Sea bream; P指导us major.

The common carp, Cyprinus carpio usually browse on the bottom in the environment and could uptake, and accumulate more pollutants (Mustafa et al., 2012). This species as a freshwater teleost is also cultured in many parts of the world and thus more likely to be exposed to different types of pollutants such as heavy metals and MNMs. Furthermore, common carp has received more attention in recent years as a model organism for toxicity studies (Raj Gupta et al., 2016; Chupani et al., 2017, 2018a, 2018b). Therefore, the present study was conducted to compare the toxicity effects of low and high concentrations of two kinds of dietary copper sources including CuSO\(_4\) and Cu-NPs on growth performance and survival, as well as blood biochemical indices and bioaccumulation of copper in different organs of common carp (C. carpio) after dietary exposure and recovery periods.

### 2. Materials and methods

#### 2.1. Copper oxide nanoparticles and characterization

Powder of CuO-NPs was purchased from US Research Nanomaterials, Inc. (3302 Twig Leaflane, Houston, TX77084). Particles were characterized using field emission scanning electron microscopy (FE-SEM; MIRA3 TESCAN, Brno, Czech Republic) coupled with energy dispersive X-ray spectroscopy (EDS). Average diameter of particles on FE-SEM images was estimated by measuring the diameter of 200 individual particles using AxioVision digital image processing software (Release 4.8.2.0, Carl Zeiss Micro Imaging GmbH, Germany).

#### 2.2. Preparation of experimental diets

A basal diet detailed by Dekani et al. (2019) was applied in the present study. Table 1 represents the proximate composition and chemical analysis of the basal diet. To prepare the experimental diets, the basal diet was supplemented with CuO-NPs or CuSO\(_4\) at two concentrations of 100 and 1000 mg kg\(^{-1}\) of dry diet. The inclusion levels of copper were chosen based on a literature review on fish requirement (Clearwater et al., 2002; Wang et al., 2018) and toxicological effects following dietary exposure to teleost fish (Berntssen et al., 1999; Kim and Kang, 2004; Shaw and Handy, 2006). At first, a premix of copper source and wheat flour was prepared and added to other components of diet. Then, a uniform paste was prepared by adding water and the mixture was extruded. Finally, the wet pellets were air-dried at room temperature. Release of copper to water during the feeding period was prevented by spraying bovine gelatin solution (10%) on the surfaces of the prepared pellets and oven-dried at 55 °C (Ramsden et al., 2009). The diet without adding copper was considered as control. The copper content of the prepared diets was measured using an ICP-OES (Spectro Arcos).

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meal</td>
<td>30</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>18</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>8</td>
</tr>
<tr>
<td>Corn meal</td>
<td>20</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>16</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>2</td>
</tr>
<tr>
<td>Fish oil</td>
<td>2</td>
</tr>
<tr>
<td>Cu free premix</td>
<td>3.5</td>
</tr>
<tr>
<td>Salt</td>
<td>0.5</td>
</tr>
</tbody>
</table>

#### 2.3. Experimental design

Specimens of common carp (C. carpio) with the average initial weight of 35.94 ± 5.35 g and average initial length of 13.75 ± 2.09 cm were obtained from a local fish farm in north of Iran. Fish (n = 500) were stocked in six fiberglass tanks (500 L) for two weeks and fed on the basal diet to apparent satiation twice a day. Thereafter, fish were individually weighted and distributed randomly into 15 tanks (500 L) at a density of 30 fish tank\(^{-1}\) in triplicate for each experimental diet. Fish were fed with supplemented diets with Cu and basal diet (without added Cu; control) three times a day at 8:00, 12:00 and 16:00 for 21 days to apparent satiation. During feeding trial, 50% of water of each tank was replaced daily with fresh water, and uneaten

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**Table 1** Chemical analysis and proximate composition of the basal diet used in the dietary experiment.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meal</td>
<td>24.51 ± 0.40</td>
</tr>
<tr>
<td>Crude fat</td>
<td>14.00 ± 1.15</td>
</tr>
<tr>
<td>Moisture</td>
<td>7.60 ± 0.71</td>
</tr>
<tr>
<td>Crude ash</td>
<td>8.59 ± 0.74</td>
</tr>
</tbody>
</table>
feeds were also siphoned. Thereafter, fish were fed on the basal diet (without Cu supplementation) for another 21 days as a depuration or recovery period. Photoperiod regime of 16:8 (light: dark) was performed during experimental period. Water quality including temperature, pH and dissolved oxygen was measured every day and recorded as 24.7 ± 0.9 °C, 7.32 ± 0.4 and 6.3 ± 0.46 mg L⁻¹, respectively. All animal manipulation procedures were done based on Animal Welfare Act and Interagency Research Animal Committee guidelines (Nickum et al., 2004).

2.4. Growth performance assessment

After 11 and 21 days of dietary exposure period as well as at the end of recovery phase, fish were starved for 24 h. Then, fish were individually anesthetized using 500 mg L⁻¹ clove powder, weighted and counted to calculate the growth performance parameters and survival rate using following equations (Fernández-Montero et al., 2018):

\[ \text{Weight gain (WG)} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Initial weight (g)}} \]

\[ \text{Condition factor (CF)} = \left( \frac{\text{Final weight (g)}}{\text{Final length (cm)}} \right)^3 \times 100 \]

\[ \text{Hepatosomatic Index (HSI)} = \left( \frac{\text{Liver weight (g)}}{\text{Body weight (g)}} \right) \times 100 \]

\[ \text{Survival rate (S)} = \left( \frac{\text{Final number of fish}}{\text{Initial number of fish}} \right) \times 100 \]

2.5. Blood biochemical assay

At the end of dietary exposure and depuration periods, ten fish were randomly sampled from each tank and anesthetized using clove powder (500 mg L⁻¹). The blood samples were taken from the caudal vein using heparinized syringes. The collected samples were centrifuged (Velocity 14R model) at 5000 rpm for 10 min to obtained plasma and stored at −80 °C up to measure the biochemical parameters. Plasma glucose was measured using an assay kit (Pars Azmoon Inc., Tehran, Iran) in an auto analyzer. Plasma potassium and sodium values were assayed based on ion selective electrode (ISE) technique using an electrolyte analyzer (Geni-Tech-G200 model). The concentrations of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) enzymes in the plasma samples were determined using a commercial kit (Pars Azmoon Inc., Tehran, Iran).

2.6. Copper bioaccumulation

To measure the accumulation of copper in gills, intestine, liver and kidney tissues, five fish were randomly selected per replicate at mid and end of dietary exposure and depuration phases and dissected to remove the mentioned organs. The sampled organs were dried for 24 h using a freeze-dryer (Dena Vacuum, FD-5005-BT). The dried sample (0.1 g) were digested using 3 mL of concentrated nitric acid (Suprapur® grade, Merck, Germany) and heated for 120 min at 100 °C on a Bain-Marie bath (Memmert, WNB 45 model) (Nemati et al., 2019). The concentration of copper in digested samples was measured using an ICP-OES (Spectro Arcos).

2.7. Statistical analysis

Data were reported as mean ± SD. Normality of the data was assayed using Kolmogorov–Smirnov test. Significant differences between means were determined using one-way analysis of variance (ANOVA), and one-way repeated measures analysis of variance (ANOVA with repeated measures), followed by the Duncan test. The Paired-Sample t-test was employed to compare the differences between two paired samples. Significant differences were accepted at the level of \( P < 0.05 \). The statistical analyses were carried out by SPSS software (Version, 19).

3. Results

3.1. Characterization of CuO-NPs

Fig. 1(a, b) shows FE-SEM and EDS analysis results of CuO-NPs powder. The analysis of elemental composition of CuO-NPs powder by EDS revealed the presence of copper and oxygen as main elements. FE-SEM micrograph proved the nanoscale sizes of particles with mean diameter of 87.6 ± 31.2 nm and size distribution ranged from 6.8 to
Table 2
The actual and nominal concentrations of copper in the experimental diets supplemented with CuO nanoparticles (CuO-NPs) and ionic Cu (CuSO₄).

<table>
<thead>
<tr>
<th>Cu dietary treatment</th>
<th>Cu concentration (mg kg⁻¹ dry diet)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nominal</td>
</tr>
<tr>
<td>Control (basal diet)</td>
<td>0⁻</td>
</tr>
<tr>
<td>CuO-NPs</td>
<td>100⁻</td>
</tr>
<tr>
<td></td>
<td>1000⁻</td>
</tr>
<tr>
<td>CuSO₄</td>
<td>100⁻</td>
</tr>
<tr>
<td></td>
<td>1000⁻</td>
</tr>
</tbody>
</table>

*The data with different letters in each row are significantly different (mean ± SD, One-sample t-test, P < 0.05).

253.7 nm.

3.2. Cu content of experimental diets

Table 2 shows the actual and nominal concentrations of copper (Cu) in prepared diets. The actual concentration of Cu in the control group was significantly higher than the nominal concentration (P = 0.001), which may be due to the presence of copper in the dietary components. In the 1000 mg kg⁻¹ of CuO-NPs dietary group, the actual concentration was significantly lower than the nominal concentration (P = 0.034). There were no significant differences between the actual and nominal concentrations in the other dietary groups (P = 0.10, 0.39, 0.081).

3.3. Growth performance and survival rate

Growth performance parameters and survival rate of fish fed on Cu-supplemented diets after exposure and recovery periods are shown in Figs. 2–5. Diet supplementation with different Cu sources decreased WG compared to the control group after 21 days of dietary exposure (P = 0.00). The lowest WG was observed in 1000 mg kg⁻¹ both in nanoparticle and ionic dietary groups (P = 0.00) (Fig. 2a). After 21 days recovery period, fish fed diets with 100 and 1000 mg kg⁻¹ of CuSO₄ had lower WG than control (P = 0.001) (Fig. 2b).

Food intake of fish fed on diets supplemented with Cu sources decreased significantly compared to the control group after the 21 days of dietary exposure (P = 0.00). Fish fed 1000 mg kg⁻¹ of CuO-NPs and CuSO₄ supplemented-diets showed the lower food intake than other dietary groups (P = 0.00) (Fig. 3a). After recovery period, the food intake of fish in Cu-supplemented diet groups was significantly lower than the control group (P = 0.00) (Fig. 3b).

Condition factor (CF) of fish had no significant differences between the experimental groups after dietary exposure and recovery periods (P = 0.02, 0.34) (Fig. 4a). Fish fed with different Cu sources showed no significant differences in hepatosomatic index (HSI) in none of experimental period (P = 0.18). HSI of fish after recovery period decreased significantly compared to 11 days dietary exposure period in each the experimental group (P = 0.023) but did not change in comparison to 21 days dietary exposure (P = 0.14) (Fig. 4b).

The survival rates of fish fed 100 mg kg⁻¹ of CuO-NPs and CuSO₄ supplemented-diets were significantly lower than other dietary groups after 21 days of dietary exposure (P = 0.003) (Fig. 5). No mortality was recorded in each experimental group after recovery period.

3.4. Biochemical analyses

The variations in plasma indices of fish fed with different Cu sources are presented in Fig. 6(a–e). Glutamate pyruvate transaminase (GPT) activity decreased in carp fed diets contained 100 and 1000 mg kg⁻¹ of CuSO₄ and 1000 mg kg⁻¹ CuO-NPs compared to the control group after dietary exposure period (P = 0.00). After recovery period, the activity of this enzyme was only higher than the control in fish fed diet with 1000 mg kg⁻¹ of CuSO₄ (P = 0.004). The GPT activity after recovery period was significantly higher than the dietary exposure period in the 100 and 1000 mg kg⁻¹ of CuSO₄ (P = 0.001, 0.002) (Fig. 6a). Fish fed on Cu-supplemented diet showed lower activity of glutamate oxaloacetate transaminase (GOT) than the control group after dietary exposure period (P = 0.008). After recovery period, the activity of this enzyme decreased significantly compared to the dietary exposure period in 100 and 1000 mg kg⁻¹ of CuO-NPs (P = 0.008, 0.002) (Fig. 6b). Plasma potassium level of carp increased after feeding diets containing 100 and 1000 mg kg⁻¹ of CuSO₄ and 1000 mg kg⁻¹ of CuO-NPs compared to the control (P = 0.035). There was no significant difference among the dietary groups after the recovery period (P = 0.1). In each dietary group, the level of plasma potassium after recovery period was lower than the dietary exposure period (P = 0.02, 0.03, 0.027, 0.003, 0.035) (Fig. 6c). Plasma sodium showed lower level in fish fed on diet with 1000 mg kg⁻¹ of CuO-NPs compared to the control (P = 0.001). After recovery period, sodium level increased in 100 and 1000 mg kg⁻¹ of CuO-NPs compared to the control.

![Fig. 2. Weight gain (WG) of common carp (C. carpio) fed diets containing 100 or 1000 mg kg⁻¹ of different Cu sources, including CuO nanoparticles (Nano) and CuSO₄ (Ion): After 21 days of dietary exposure (a) and after 21 days of recovery period (b). Bars with different letters are significantly different (mean ± SD, ANOVA, P < 0.05).](image-url)
Fig. 3. Food intake of common carp (C. carpio) fed diets containing 100 or 1000 mg kg$^{-1}$ of different Cu sources, including CuO nanoparticles (Nano) and CuSO$_4$ (Ion): After 21 days of dietary exposure (a) and after 21 days of recovery period (b). Bars with different letters are significantly different (mean ± SD, ANOVA, $P < 0.05$).

Fig. 4. Condition factor (CF) (a) and hepatosomatic index (HSI) (b) of common carp (C. carpio) fed diets containing 100 or 1000 mg kg$^{-1}$ of different Cu sources, including CuO nanoparticles (Nano) and CuSO$_4$ (Ion). Mid: after 11 days of dietary exposure, End: after 21 days of dietary exposure, Rec: after 21 days of recovery period. The bars with different letters in each Cu source are significantly different (mean ± SD, ANOVA, $P < 0.05$).
the dietary exposure phase (P = 0.003). The Cu level in 1000 mg kg \(^{-1}\) of CuO-NPs and CuSO\(_4\) was only higher in 100 mg kg \(^{-1}\) of CuO-NPs and CuSO\(_4\) than other dietary groups, which was consistent with the results of the weight gain of fish. Bernstesn et al. (1999) reported that growth of Atlantic salmon (Salmo salar) fry significantly decreased after feeding with diet containing > 467 mg Cu (CuSO\(_4\)) kg \(^{-1}\) dry diet for 12 weeks. The finding of another study also revealed that feeding grey mullet, Chelon labrosus with diet supplemented with CuSO\(_4\) at level of 2400 mg Cu kg \(^{-1}\) dry diet for 10 weeks caused decreased food intake and growth (Baker et al., 1998). In contrast, Cu in the form of nano CuO at 2 and 4 mg kg \(^{-1}\) of diet improved the growth of red sea bream, Pagrus major (El-Imrani et al., 2016a, 2016b) and Russian sturgeon, Acipenser gueldenstaedti (Wang et al., 2018), respectively. Based on the results of present study, the WG of CuSO\(_4\) dietary groups was lower than CuO-NPs dietary groups after feeding with non-supplemented diet during recovery period, suggesting that Cu in form of CuSO\(_4\) had higher inhibitory effect on growth, so that the food intake of fish was lower in 1000 mg kg \(^{-1}\) of CuSO\(_4\) than other Cu-dietary groups. Whole-body responses such as CF and HSI were not affected by different Cu-supplemented diets. In addition, the results demonstrated that the survival rates of carp in 1000 mg kg \(^{-1}\) of CuO-NPs and CuSO\(_4\) were higher than 100 mg kg \(^{-1}\) of Cu-dietary groups, likely due to lower food intake and consequent the reduction of copper toxicity.

Glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) enzymes are known as indicators or general biomarkers of stressful conditions and environmental pollution (El-Shehawi et al., 2007; Ozmen et al., 2008; Vutukuru et al., 2007). It is well known that these enzymes form links between the metabolism of amino acids, carbohydrates and fats (Rudha Abbas et al., 2011). The GOT and GPT can be found normally in tissues such as liver, kidney, heart and skeletal muscle and damage to tissue cells may result in liberation of these enzymes in blood circulation (Jeneya et al., 1992; Rather, 2015). Therefore, activation and inhibition of the GOT and GPT in tissues and blood can be used as biochemical indices to assess the heath of fish in aquatic ecosystems contaminated with various pollutants. Some studies have reported an increase in the GOT and GPT activity in serum of Sparus aurata (Vaglio and Landriscina, 1999), Clarias gariepinus (Al-Otaibi et al., 2019), C. carpio (David et al., 2010) exposed to different toxicants. Rather (2015) found an increase in the serum GOT and GPT levels in catfish, Clarias batrachus exposed to sublethal concentrations of carbaryl and parathion for 28 days. The results of another study revealed that dietary Cu increased the GOT and GPT activities in the serum of juvenile rockfish, S. schlegeli with increasing time and exposure dose (Kim and Kang, 2004). In contrast, the finding of the present study showed that the GOT level in plasma of carp fed on Cu-supplemented diets for 21 days decreased significantly compared to the control. The level of GPT also decreased in fish fed on diets containing 100 and 1000 mg kg \(^{-1}\) of CuSO\(_4\) and 1000 mg kg \(^{-1}\) of CuO-NPs compared to the control. It is believed that heavy metals such as silver can react with proteins, bind to thiol groups of enzymes, and inactivate them (Elechiguerra et al., 2005; Raffi et al., 2008). Rudha Abbas et al.
Fig. 6. Glutamate pyruvate transaminase (GPT) (a), glutamate oxaloacetate transaminase (GOT) (b), potassium (c), sodium (d) and glucose levels in plasma of common carp (C. carpio) fed diets containing 100 or 1000 mg kg\(^{-1}\) of different Cu sources, including CuO nanoparticles (Nano) and CuSO\(_4\) (Ion). End: after 21 days of dietary exposure, Rec: after 21 days of recovery period. Bars with different lowercase letters in each period are significantly different (mean ± SD, ANOVA, \(P < 0.05\)). Bars with different capital letters in each Cu source are significantly different (mean ± SD, Paired-Sample \(t\)-test, \(P < 0.05\)).
sources, including CuO nanoparticles (Nano) and CuSO4 (Ion). Mid: after 11 days of dietary exposure, End: after 21 days of dietary exposure, Rec: after 21 days of recovery period. Bars with different lowercase letters in each period are significantly different (mean ± SD, ANOVA, P < 0.05). Bars with different capital letters in each Cu source are significantly different (mean ± SD, ANOVA, P < 0.05).

Fig. 7. Tissue copper burden in gill (a) intestine (b), liver (c) and kidney (d) of common carp (C. carpio) fed diets containing 100 or 1000 mg kg⁻¹ of different Cu sources, including CuO nanoparticles (Nano) and CuSO₄ (Ion). Mid: after 11 days of dietary exposure, End: after 21 days of dietary exposure, Rec: after 21 days of recovery period. Bars with different lowercase letters in each period are significantly different (mean ± SD, ANOVA, P < 0.05). Bars with different capital letters in each Cu source are significantly different (mean ± SD, ANOVA, P < 0.05).

(2011) reported that silver and gold nanoparticles colloids had inhibitory effects on the GOT and GPT activities in human serum in a dose-dependent manner. Presumably, copper in the both Nano and ionic-forms like other heavy metals inhibited the activity of GPT and GOT through binding to thiol groups of these enzymes. The inhibitory effect of 100 mg Cu kg⁻¹ of diet in ionic form on the GPT activity was significantly higher than Nano-form. The both forms of copper at two different concentrations had the same inhibitory effect on the GOT activity. In the present study, the recovery period and feeding with non-supplemented diet had positive effect on the GOT and GPT activity, so that the levels of these enzymes in dietary groups showed no significant differences compared to the control group.

In lower vertebrate including fish, sodium uptake occurs via sodium pathway that contained apical, H⁺-ATPase-coupled Na⁺ channel and a basolateral Na⁺/K⁺-ATPase extruding sodium from the epithelial cells to the blood plasma (Fenwick et al., 1999; Grosell and Wood, 2002). In the present study, the plasma Na⁺ level decreased in 1000 mg kg⁻¹ of CuO-NPs dietary group compared to the control, suggesting inhibition of intestinal Na⁺/K⁺-ATPase activity and disruption of intracellular sodium influx into the blood. In 1000 mg Cu kg⁻¹ diet, the reduction of plasma Na⁺ level in CuO-NPs dietary group was higher than CuSO₄. In addition, the increased plasma K⁺ level in the all dietary groups except 100 mg kg⁻¹ of CuO-NPs indicating copper toxicity associate with ionoregulatory disturbance at the plasma level. Waterborne exposure to 100 μg l⁻¹ of Cu-NPs in juvenile rainbow trout (O. mykiss) showed depletion of plasma Na⁺ and decreased intestinal Na⁺/K⁺-ATPase activity (Shaw et al., 2012). Griffitt et al. (2007) also reported the depletion of plasma Na⁺ and inhibition of the branchial Na⁺/K⁺-ATPase activity in zebrafish (Danio rerio) exposed to 0.25 and 1.5 mg L⁻¹ of Cu-NPs. After the recovery period, the plasma Na⁺ level in 1000 mg kg⁻¹ of CuO-NPs group increased and reached to the control value. The plasma potassium levels in the all Cu-supplemented diet groups returned to the control value after recovery phase.

In this study, different concentrations of two forms of Cu sources did not affect the plasma glucose level after dietary exposure periods. The glucose level was also same in the all-dietary groups after recovery period. Mohseni et al. (2014) showed that the serum glucose level had no significant differences between juvenile beluga, Huso huso fed on diet containing 195 mg Cu (CuSO₄·5H₂O) k⁻¹ of diet and those fed on diet supplemented with 1 mg Cu k⁻¹ of diet. The results of another study also revealed that dietary Cu exposure at concentrations of 0 to 500 mg k⁻¹ of dry diet for 60 days had no significant effects on glucose serum concentrations in juvenile rockfish, S. schlegelti (Kim and Kang, 2004).

In the present study, the order of copper accumulation in organs after dietary exposure period was dose-dependent. In 100 mg k⁻¹ of CuSO₄ and CuO-NPs dietary groups, the highest Cu accumulation was observed in liver followed by intestine, gill and kidney. In fish, Cu metabolism mainly occurs in the liver organ (Lindh et al., 2019). The liver accumulates a large proportion of copper (Grosell et al., 2001).
Lindh et al. (2019) showed that copper mainly accumulated in the liver of rainbow trout (Onchorhyncus mykiss) injected intraperitoneally with non-lethal doses of either CuSO4 or CuNPs. In the case of blugas, Huo huso copper accumulated mostly in the liver, followed by the intestine, gill, muscle and kidney after feeding with diets supplemented with 1–195 mg kg\(^{-1}\) of CuSO4 for 12 weeks (Mohseni et al., 2014). Kamunde et al. (2002) also reported that the Cu liver content in rainbow trout (O. mykiss) fed 282 mg Cu kg\(^{-1}\) diet was 33-fold higher than the control group. In 1000 mg kg\(^{-1}\) of CuSO4 and CuO-NPs dietary groups, the order of Cu accumulation changed to intestine > liver > kidney > gill. Lindh et al. (2019) explained that the elevated Cu concentration in the intestine of rainbow trout (O. mykiss) exposed to dietary CuNPs is likely due to binding of NPs to mucus and their intestinal uptake. Dietary exposure of Atlantic salmon (S. salar) to 500 and 700 mg Cu kg\(^{-1}\) diet resulted in the accumulation of 50% of whole-body Cu in intestine tissue (Lundebye et al., 1999).

In 100 mg Cu kg\(^{-1}\) diet, the gills copper content in CuSO\(_4\) group was significantly higher than CuO-NPs group. Isani et al. (2013) also found that gill Cu content was significantly higher in rainbow trout (O. mykiss) injected intraperitoneally with CuSO\(_4\) than CuO nanoparticles. The accumulation of Cu in the gills was same in the 1000 mg kg\(^{-1}\) of CuSO\(_4\) and CuO-NPs dietary groups. After recovery phase, the gill copper content only in 1000 mg kg\(^{-1}\) of CuSO\(_4\) was higher than control, indicating the lower Cu depuration rate in this dietary group.

In the intestine, liver and kidney organs, the Cu accumulation level in 1000 mg kg\(^{-1}\) of CuSO\(_4\) dietary group was significantly higher than CuO-NPs dietary groups. The uptake of Cu may be occurred through the epithelial cells of intestine (Lindh et al., 2019). Opposite to ions, NPs are too large to be taken up by ion transporter; possible route of uptake is endocytosis (Shaw and Handy, 2011). According to the results of accumulation of copper in intestine, the uptake of Cu probably by the intestine cells in ionic form was greater than that of the Nano-form. In the kidney tissue, the accumulation of the both Cu forms was observed at concentration of 1000 mg kg\(^{-1}\) of diet. In 100 mg Cu kg\(^{-1}\) diet, Cu accumulated only in the ionic form in the kidney.

Feeding carp with the basal diet (without adding Cu) during recovery period resulted in decreasing intestine and kidney Cu content. In contrast, liver Cu content did not decreased in the Cu-supplemented groups compared to the control and this value in CuSO\(_4\) dietary groups was significantly higher than CuO-NPs groups after recovery period. These results revealed that copper mostly accumulated in liver of carp and Cu depuration rate of nanoparticles form was higher than ionic form. Similarly, Shaw and Handy (2006) reported that intestinal and branchial Cu levels of Nile tilapia, O. niloticus fed on Cu-loaded diet (2000 mg kg\(^{-1}\) dry diet) for 42 days decreased compared to the control after 21 days of recovery period, while liver Cu content increased approximately 1.7-fold.

5. Conclusion

The present study provides detailed effects of dietary nanoparticles and ionic Cu sources on survival, growth performance and some blood biochemical indices as well as bioaccumulation of Cu in target organs of common carp (C. carpio). The findings of this study revealed that the food intake of fish in 100 mg kg\(^{-1}\) of CuO-NPs and CuSO\(_4\) groups was higher than other Cu-supplemented diet groups, which resulted in higher fish mortality in these dietary groups during the 21 days of exposure period. In contrast, the fish exposed to 1000 mg kg\(^{-1}\) of CuO-NPs and CuSO\(_4\) reduced their food intake. Therefore, they showed the higher survival rate but the less weight gain. However, the both Cu sources at concentrations of 100 and 1000 mg kg\(^{-1}\) diet induced alterations of the plasma GOT, GPT, sodium and potassium levels, indicating the toxicity effects of both Cu sources on carp fish. The exposure of fish to Cu-supplemented diets also resulted in the copper accumulation in target organs in a dose-dependent manner. Cu accumulated mostly in the intestine tissue in 1000 mg Cu kg\(^{-1}\) diet, while the liver of fish fed on diets containing 100 mg Cu kg\(^{-1}\) diet showed the highest Cu content. According to the results, the recovery period had positive effect on the reduction of dietary toxicity of both Cu sources, so that the levels of most of biochemical indices and tissues Cu content decreased or returned to the control value after this period.

Declaration of competing interest

The authors have no conflicts of interest to declare.

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